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Proceedings of the 7th New Drug Seminar:

Nitrosoureas

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The Secretary of Health, Education, and Welfare has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through November 30, 1976.

Seventh New Drug Seminar on the Nitrosoureas

Division of Cancer Treatment
National Cancer Institute

Editor: Stephen K. Carter

December 15–16, 1975
Washington, DC

Introduction

The Division of Cancer Treatment (DCT), NCI, which evolved out of the old Chemotherapy Program, still has as one of its major responsibilities the development and clinical testing of new anticancer drugs. For many years, the DCT has attempted to inform practicing physicians about investigational drugs which have reached the point of commercial applicability. One of the tools utilized to accomplish this has been the New Drug Seminar in which a group of leading scientists who have played significant roles in investigating a given compound are brought together to summarize the available data on the drug for interested clinicians. In the past, New Drug Seminars have been held on cytosine arabinoside, procarbazine, mithramycin, mitotane, bleomycin, adriamycin, and DTIC. The 7th New Drug Seminar, published in this issue, was devoted to the nitrosoureas, with particular emphasis on BCNU and CCNU which will soon be commercially available. The nitrosoureas represent one of the major successes of the DCT's Drug Development Program, and it is hoped that extensive and interesting data presented in this seminar will be of value to the practicing clinician as he begins to integrate these compounds into standard therapy.

On behalf of the DCT, I would like to thank all of the individuals who participated in the seminar and submitted papers for publication.

Stephen K. Carter

Early History and Development of the Nitrosoureas

Saul A. Schepartz[1,*]

The nitrosoureas represent one of the most significant groups of anticancer agents that have been developed since the initiation of the National Cancer Institute's drug development effort in 1955. As will be illustrated in this and other papers in this issue, these compounds possess a very high degree of activity in experimental tumor systems and have demonstrated their usefulness in a variety of human malignancies. They also represent one of the most interesting developmental stories in terms of the interrelationship between empiric observations and rational drug design. In this report I will present a brief history of the early development of these agents, leaving the more detailed presentations of more recent information to the other papers in this seminar.

The first compound in the series was 1-methyl-3-nitro-1-nitrosoguanidine (fig 1).[2] In accordance with our policy to obtain a variety of new chemicals for screening in our experimental tumor systems, members of our staff collected this compound from one of the stockrooms at the National Institutes of Health at the very beginning of our program. It had, in fact, been synthesized in 1947 with no biologic rationale in mind (1). In the course of testing against the L1210 leukemia, this compound demonstrated a reproducible but not outstanding ability to increase the lifespan of leukemic animals (2). Representative data obtained in this system (table 1) illustrate that 1-methyl-3-nitro-1-nitrosoguanidine was active in the L1210 leukemia via the ip route of injection but not by either sc or oral administration. It barely met our criteria for development toward clinical trial (50% increase in lifespan [ILS] of treated animals); therefore, it was of moderate interest at the time, particularly in view of its lack of activity by routes other than ip. It was, however, selected for development in April 1958 and this development occurred over a period of approximately 3 1/2 years from its submission to the NCI in 1956 to the initiation of rather abbreviated clinical studies in 1960. The reason for the rather abbreviated study was that a much better compound was developed through the L1210 leukemia screen shortly thereafter. This will be described later. Once the initial activity with 1-methyl-3-nitro-1-nitrosoguanidine had been observed and confirmed, active efforts were undertaken in contract synthesis laboratories to develop improved analogs through rational design. These efforts took place both at the Stanford Research Institute, Palo Alto, Calif, and the Southern Research Institute, Birmingham, Ala. 1-Methyl-1-nitrosourea[3] was a relatively simple and logical analog submitted to the program rather early in the course of this development by the Southern Research Institute investigators; its structure is illustrated in figure 1. Actually, this compound had been in the chemical literature since 1888 (3). As demonstrated in table 1, this compound exhibited somewhat greater activity against the L1210 leukemia when administered ip (87% ILS versus 50% ILS for the parent compound). In addition, it also demonstrated significant and essentially equal activity when administered sc or orally. Of even greater interest at the time, however, was the fact that 1-methyl-1-nitrosourea was effective against L1210 leukemia when the tumor was implanted intracerebrally (ic) (4) (table 2); thus, it had the apparent ability to cross the so-called blood-brain barrier and became of much greater interest as a result of this observation. 1-Methyl-1-nitrosourea, which had been submitted initially in May 1958, was selected for development in January 1960 at the same time 1-methyl-3-nitro-1-nitrosoguanidine had entered clinical trial. Preclinical development of 1-methyl-1-nitrosourea proceeded from 1960 until late 1962. This drug never entered clinical studies in the US, however, for two primary reasons: (1) the compound was exceedingly unstable chemically and was, therefore, very difficult to handle for widespread clinical studies; and (2) as the preclinical development reached its completion a better compound became available. This third compound, BCNU, was submitted to the NCI in April 1962 (fig 1).[4] BCNU was one of a rather large

[1]Drug Research and Development Program (DR&DP), Division of Cancer Treatment (DCT), National Cancer Institute (NCI), Bethesda, Md.

[2]1-Methyl-3-nitro-1-nitrosoguanidine: NSC-9369; CAS reg. No. 70-25-7.

*Reprint requests to: Dr. Saul A. Schepartz, DCT, NCI, Bldg 31, Rm 3A51, National Institutes of Health, Bethesda, Md 20014.

[3]1-Methyl-1-nitrosourea: NSC-23909; CAS reg. No. 684-93-5.

[4]BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-.

TABLE 1.—Effect of nitrosoureas against L1210 leukemia

Nitrosourea	Route of injection	Daily dose: Day 1 to death (mg/kg/injection)	% ILS*
1-Methyl-3-nitro-1-nitrosoguanidine	ip	40	5
	ip	20	50
	ip	10	47
	ip	5	38
	sc	40	21
	sc	20	23
	sc	10	15
	sc	5	14
	oral	160	Toxic
	oral	80	Toxic
	oral	40	8
	oral	20	23
1-Methyl-1-nitrosourea	ip	20	39
	ip	15	67
	ip	10	87
	ip	5	27
	sc	25	3
	sc	17	26
	sc	11	74
	sc	7	63
	sc	5	38
	oral	119	0
	oral	77	31
	oral	49	51
	oral	33	27

*Increase in mean lifespan above controls.

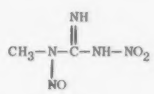
TABLE 2.—Effect of nitrosoureas against ic L1210 leukemia*

Nitrosourea	Route of injection	Schedule	Dose (mg/kg/injection)	% ILS†
1-Methyl-1-nitrosourea	ip	Day 1 only	144	Toxic
	ip	Day 1 only	72	52
	ip	Day 1 only	36	10
	ip	Day 1 only	18	2
BCNU	ip	Day 1 to death	50	50 (2/10 cures)
	ip	Day 1 to death	25	163 (7/10 cures)
	ip	Day 1 to death	10	35
	oral	Day 1 to death	50	138 (7/10 cures)
	oral	Day 1 to death	25	161 (7/10 cures)
	oral	Day 1 to death	10	34

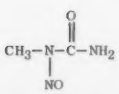
*10^1 cells implanted ic.
†Increase in mean lifespan above controls.

number of new analogs synthesized by the Southern Research Institute (5). In October 1962, it was selected for development because of striking activity in the L1210 leukemia system (6). As shown in table 3 BCNU possesses substantial activity against the L1210 leukemia when administered either ip or orally. This activity is far greater than was observed with 1-methyl-1-nitrosourea. Although not shown in the specific data presented in table 3, BCNU consistently produces long-term survivors among animals with L1210 leukemia when treated under optimal dosage schedules. More detailed data of this nature will be presented in other papers in this issue. With regard to activity against the ic implanted L1210 leukemia (table 2), BCNU again substantially exceeded the effects observed with 1-methyl-1-nitrosourea, providing not only significant increases in survival but a large percentage of long-term survivors or "cures." In view of the substantial activity of BCNU against L1210 leukemia and a variety of other experimental systems as well (7), the compound was selected for clinical development in October 1962. The time of BCNU development was greatly reduced since clinical trials were initiated approximately 6 months from the time the drug was submitted to the NCI.

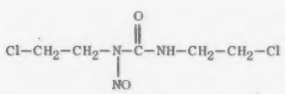
In spite of significant toxicity encountered in the course of clinical studies, BCNU therapy has been found to be of substantial benefit, as will be discussed in other papers in this issue. By the late 1960s it became clear that BCNU, and possibly other nitrosoureas as well, should be made available to the general public. As a result of this decision an effort was made to select competitively a pharmaceutical company to prosecute the New Drug Application with the Food and Drug Administration and initiate marketing of the drug to the general public. A contract for this purpose was signed with Bristol Laboratories, Syracuse, NY, in September 1972, and it is hoped that BCNU will be made available to the public in the very near future.



1-Methyl-3-nitro-1-nitrosoguanidine



1-Methyl-1-nitrosourea



BCNU

FIGURE 1.—Structures of 3 nitrosoureas.

TABLE 3.—Effect of BCNU against ip L1210 leukemia*

Route of injection	Daily dose: Day 1 to death (mg/kg/injection)	% ILS†
ip	25	27
ip	12.5	82
ip	6.25	198
ip	3.12	130
ip	1.56	27
oral	16	73
oral	8	187
oral	4	49
oral	2	29

*10^5 cells implanted ip.
†Increase in mean lifespan above controls.

As stated earlier, more detailed discussions of the preclinical and clinical data will be presented in other papers. The purpose of this brief report is to illustrate the early history of these compounds and to attempt to point out the valuable interactions that can occur between empiric observations and rational design. With this particular series of compounds, the observation of moderate but not outstanding activity in a new chemical structure encouraged rational design to be pursued which finally resulted in a series of highly active and, we hope, highly useful antitumor agents. We believe that empiric observations provide an essential input of new information to those engaged in rational design, and therefore, that both types of approaches should be pursued vigorously.

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Chemistry and Structure-Activity Studies of the Nitrosoureas[1]

John A. Montgomery[2,3,*]

SUMMARY

The *N*-nitrosoureas are the most active of a variety of *N*-nitroso compounds tested against L1210 leukemia in mice. Structure-activity studies show that the *N*-(2-chloro(or fluoro)ethyl)-*N*-nitrosoureido grouping is necessary for high-level activity in this test system, but a variety of carrier groups can be attached to the *N'*-nitrogen of the urea. For high-level activity against solid tumors in rodents, eg, the Lewis lung adenocarcinoma, the *N'*-substituent must be a cyclohexane ring, and the most active of these compounds are substituted at position 4 of the ring. The chemistry involved in the synthesis and reactions of the nitrosoureas is described. The possible relationship of the chemically reactive species generated by the decomposition of the nitrosoureas under in vivo conditions to biologic activity is discussed.

[Cancer Treat Rep 60:651–664, 1976]

A number of types of *N*-nitroso compounds have been evaluated for their activity against L1210 leukemia (1–7) (table 1). The first of these, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (I), which gave about a 30% increase in lifespan (ILS) (1), barely met the requirements to be classed as active by the NCI protocol. *N*-methyl-*N*-nitrosourea (MNU) (II) had an ILS of 109% and *N*,*N'*-dimethyl-*N*-nitrosourea (III) an ILS of 61% (4). The thiourea (IV) (4) and the nitrososulfonamides (V) (2) are all inactive in this test system. The 2-chloroethyl group imparts a higher degree of activity to *N*-nitroso compounds than the methyl group, but comparisons in that series (VI–XI) also show the clear superiority of the nitrosoureas (VII and X) (4,6,7) to the nitroguanidine (VI) (1,3), the urethane (VIII) (3), the amides (IX) (5), and the sulfonamide (XI) (3). Of particular note is the contrast in activity of the tosylurea (X) (7) and the tosylamide (XI) (3).

All of the nitrosoureas used in this study were prepared by the nitrosation of the corresponding ureas (XIV) (fig 1) (4–7). The nitrosating agents used were anhydrous sodium nitrite, dinitrogen trioxide, and dinitrogen tetraoxide; in most cases they were used in the presence of an acid, hydrochloric, sulfuric, or anhydrous formic. These combinations generate the true nitrosating agents in situ: nitrous acid, nitrosyl chloride, and formyl nitrite. The ureas (XIV) to be nitrosated were prepared by the reaction of an isocyanate (XII), usually methyl or 2-chloroethyl, with an amine (XIII). In some cases the isocyanate was generated in situ from the appropriate nitrosourea (XVII). For example, *N'*-(2-chloroethyl)-*N*-methyl-*N*-nitrosourea is a good source of 2-chloroethyl isocyanate (4). Nitrosation of an unsymmetric urea (XIV) can give two isomeric nitrosoureas (XV and XVI), but appropriate conditions can usually be found for each structure that allow some degree of control of the position of nitrosation. For example, dry sodium nitrite powder added to a solution of *N*-(2-chloroethyl)-*N'*-cyclohexyl(or phenyl)urea in anhydrous formic acid gave predominantly one isomer (table 2), whereas nitrosation in aqueous formic acid gave random nitrosation. It is apparent from this table that nitrosation of monosubstituted ureas (XIVa) results in the isolation of products nitrosated exclusively on the nitrogen bearing the alkyl or aryl group to give XVa. If the urea is unsymmetric and contains two protons on both carbons α to the nitrogens, then a mixture of the two possible ureas (XVb and XVIb) is usually formed regardless of the conditions used. An excep-

[1]Supported by contract N01-CM-43762 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[2]Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Ala.

[3]I am indebted to Drs. T. P. Johnston and G. P. Wheeler for helpful discussions and to Drs. Wheeler and W. M. Shannon for the use of unpublished data.

**Reprint requests to:* Dr. John A. Montgomery, Southern Research Institute, 2000 9th Ave S, Birmingham, Ala 35205.

Table 1. —Activity of Various N-Nitroso Compounds vs. Leukemia L1210 (10^5 Cells, IP)

	RN(NO)R'		Treatment		
	R	R'	Schedule	% ILS *	Ref
I	Me	$C(=NH)NHNO_2$	chronic	30	1,3
II	Me	$CONH_2$	chronic	109	2,4
III	Me	CONHMe	chronic	61	4
IV	Me	CSNHMe	chronic	I	4
V	Me	SO_2R''	chronic	I	3
VI	$Cl(CH_2)_2$	$C(=NH)NHNO_2$	chronic	129	3
VII	$Cl(CH_2)_2$	CONHR''	Day 1 only	10/10 †	4,6,7
VIII	$Cl(CH_2)_2$	CO_2Et	chronic	I	3
IX	$Cl(CH_2)_2$	COR''	chronic	I to 65	5
X	$Cl(CH_2)_2$	$CONHSO_2C_6H_4Me$	Day 1 only	4/6 †	7
XI	$Cl(CH_2)_2$	$SO_2C_6H_4Me$	chronic	I	3

* Increase in lifespan.

† Cures/total number of animals.

tion to this statement is shown on the next line of table 2 where other groups in competition with methyl (as in XIVc) do not direct to their side of the molecule, ie, in the presence of a methyl group, nitrosation tends to take place on the nitrogen attached to the methyl group to give XVc exclusively. Similarly, if branching occurs on the α carbon in the alkyl group attached to one nitrogen, nitrosation on the other side is favored and becomes exclusive in certain cases; this can be seen from the next three structural types (XIVd–f). However, in the case of the six-membered rings, if the ring contains a double bond (XIVg) then a significant quantity of the isomer resulting from nitrosation on that side (XVIg) is formed. If the alkyl group has one methyl group and one hydrogen (XIVh) on the α carbon, significant amounts of that isomer (XVIh) are also formed. The same thing is true if the ring is five- or seven-membered (XIVi and XIVj) rather than six-membered. It is apparent from these results that steric factors control the position of nitrosation. Furthermore, in the case of a structure such as XIVf, if the nitrosating agent used gives a mixture of isomers, this mixture can be rearranged by dissolving it in either anhydrous formic acid or concentrated hydrochloric acid. This shows that under

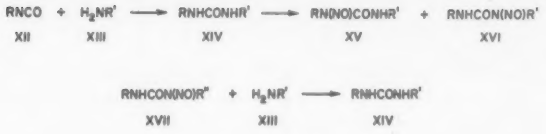
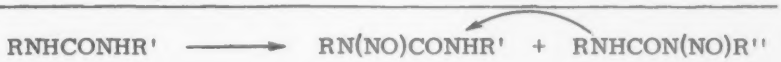


FIGURE 1

equilibrium conditions isomer formation is governed by the thermodynamic stabilities of the two products, the less stable converting into the more stable under appropriate conditions. There are limitations, therefore, on the structural types of nitrosoureas that can be formed under normal nitrosation conditions.

The limitations outlined above are important since variations in groups attached to both the nitrosated and unnitrosated nitrogens of the nitrosoureas have a profound effect on the activity of these compounds against L1210 leukemia (tables 3–7). An evaluation of a number of simple congeners of MNU (table 3) showed that all of these variations were inferior to MNU except *N*-(2-chloroethyl)-*N*-nitrosourea (CNU, NSC-47547), which was significantly better, and in later studies provided cures of the disease on the optimal treatment regimen. Table 4 relates the activity of a number of disubstituted ureas with a variety of substituted alkyl or aryl groups: all of these compounds are inferior to MNU or CNU, even those containing groups on the

Table 2.—The Nitrosation Reaction



Designation	R	R'	Approximate Isomer Ratio (%)	
a	Alkyl or Aryl	–H	100	0
b	$Cl(CH_2)_2$–	$-(CH_2)_2F$ *	50	50
c	Me–	$-(CH_2)_2Cl$	100	0
d	$Cl(CH_2)_2$–	$-C(Me)_2CH_2-C_6H_5$	100	0
e	$Cl(CH_2)_2$–		100	0
f	$X(CH_2)_2$–	six-membered ring, except	100	0
g	$Cl(CH_2)_2$–		95	5
h	$Cl(CH_2)_2$–	$-CH(Me)(CH_2)_4Me$	85-90	10-15
i	$Cl(CH_2)_2$–		75-90	10-25
j	$Cl(CH_2)_2$–		80	20

* In HCl.

β carbon electronically similar to the chloro or fluoro groups. On the other hand, with the exception of the iodo compound, all of the *N*,*N*′-bis(2-haloethyl)-*N*-nitrosoureas are equal or superior to CNU, at least against L1210 leukemia implanted ip (table 5). The bromo compounds, however, were both inactive against the ic form of the disease, probably as a result of their chemical instability. The high level of activity of the bis(2-haloethyl)nitrosoureas led to a study of the effects of variations of this type moiety on activity (table 6). Substitution of the chlorine-bearing carbon with a methyl group (NSC-95465 and NSC-95460) greatly reduces the effectiveness on a weight basis and also reduces its maximal effectiveness. Extension of the 2-chloroethyl group by a methylene group, producing the 3-chloropropyl group, results in complete loss of activity (NSC-81701) as did the other modifications shown in the table. These results directed efforts to the synthesis and evaluation of *N*-(2-chloro(or fluoro)ethyl-*N*-nitrosoureas bearing a variety of groups on the unnitrosated nitrogen. The aryl derivatives of *N*-(2-chloroethyl)-*N*-nitrosourea were in general less effective than the alicyclic derivatives against ip L1210 leukemia and were almost all ineffective against the ic form of the disease. The most active compounds resulting from this work (as judged by the L1210 system) are listed in table 7 along with their cytotoxicity to KB cells, which does not correlate with L1210 activity, and their log P values (*see* below). Note that the three compounds under clinical investigation today, BCNU (NSC-409962), CCNU (NSC-79037), and methyl-CCNU (NSC-95441) are the last three compounds in the table. Figure 2 represents an attempt to generalize the structural requirements for high-level activity. Most of these structures do, in fact, contain a saturated five- or six-membered ring, and the importance of this structural feature is emphasized by relatively low-level activity of the open-chain analog of methyl-CCNU and the cyclohexenyl analog of CCNU. Other carrier groups providing highly active compounds include the 2-haloethyl groups and certain carboxylic acids.

Hansch et al (8) have suggested that the activity of a limited group of nitrosoureas against ic implanted L1210 leukemia could be correlated with the logarithm of their partition coefficients (P) determined in the octanol-water system, using as their biologic endpoint (C) a 75% ILS. We have now compared about 80 nitrosoureas in a similar manner using a 50% cure rate of ip implanted L1210 leukemia (10^5 cells) as the biologic endpoint. The results are shown in figure 3. There is clearly a parabolic relationship between the log 1/C and log P, in spite of the fact that the highly active nitrosoureas vary widely in their log P values (table 7). Unfortunately, a similar parabolic relationship exists between the log 1/LD10 (as a measure of toxicity) and log P (fig 4); the apexes of these parabolas occur at essentially the same log P value (fig 5) indicating that there is no separation of activity and toxicity based on partition coefficients, so that an ideal log P value cannot be identified to use as a guide in designing compounds with greater selectivity in the L1210 system.

The large number of compounds highly active against L1210 leukemia ($\geq$ 50% cures at $\leq$ LD10) prompted a search for another test system more discriminating in its response to the nitrosoureas that might better predict solid tumor activity in man. One such system appears to be the Lewis lung carcinoma in mice. A number of the nitrosoureas most active against L1210 leukemia have been evaluated against both the early and the advanced disease. In general, even though there are notable exceptions, the compounds most active against the early tumor are most active against the established disease. All of the highly active compounds contain a cyclohexane ring, and most of them are substituted at the 4 position of the ring with a substituent *trans* to the *N*-(2-chloroethyl)-*N*-nitrosoureido function (table 8). The degree of dependence of activity on the stereochemistry of certain compounds is illustrated by the low level of activity of CCNU and *cis*-methyl-CCNU compared to the high level of activity of *trans*-methyl-CCNU. This same relationship exists for the *N*,*N*″-(1,4-cyclohexanediyl)bis[*N*′-(2-chloroethyl)-*N*′-nitrosourea]s (NSC-93372 and NSC-143667), but oddly enough, not for the 4-[[[(2-chloroethyl)nitrosoamino]carbonyl]amino]cyclohex-

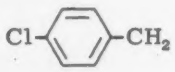
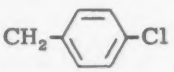
Table 3.—N-Alkyl-N-nitrosoureas (simple congeners of MNU) vs. Leukemia L1210 (10^5 Cells, IP)

R_x: IP, chronic

$RN(NO)CONH_2$		Optimal dose,	
NSC No.	R	mg/kg/day	% ILS *
23909	Me	12	109
45403	Et	50	34
48389	CH_2=$CHCH_2$	100	32
46792	n-Pr	100	30
45639	n-Bu	200	29
67523		—	inactive
71911		—	inactive
47547	$Cl(CH_2)_2$	0.9	157

* Percent increase in lifespan.

Table 4. —Activity of various 1,3-disubstituted nitrosoureas against Leukemia L1210 (10^5 Cells, IP), excluding 3-substituted 1-(2-haloethyl)-1-nitrosoureas

RN(NO)CONHR'			Optimal dose,		
NSC No.	R	R'	mg/kg	% ILS *	R_x (IP)
72721	$NC(CH_2)_2$	$(CH_2)_2CN$	150	55	single dose
409935	Me	$(CH_2)_2Cl$	150	46	chronic
89402	$MeO(CH_2)_2$	$(CH_2)_2OMe$	375	45	single dose
82190	Et	Et	400	44	single dose
75941			300	27	chronic
74255	EtO_2CCH_2	$(CH_2)_2Cl$	> 100	> 26	chronic
103525	$CH_2=CHCH_2$	$CH_2CH=CH_2$		inactive	
91742	CF_3CH_2	CH_2CF_3		inactive	
74705	$HO_2C(CH_2)_3$	$(CH_2)_3CO_2H$		inactive	
80590				inactive	
82186				inactive	
80635				inactive	

* Percent increase in lifespan.

Table 5. —1,3-Bis(2-haloethyl)-1-nitrosoureas vs. Leukemia L1210

R_x: Day 1, IP

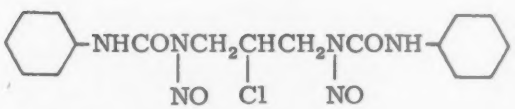
$XCH_2CH_2N(NO)CONHCH_2CH_2X$		IP, 10^5 Cells		IC, 10^4 Cells	
NSC No.	X	Log Kill	% Cures	Log Kill	% Cures
91728	F	6	50—60	5	60
409962	Cl	6	0-100	5	0-100
93184	Cl(F)	6	90-100	5	50—90
82183	Cl(Br)	6	100	0	0
82189	Br	5	20	0	0
81166	I	Inactive			

anecarboxylic acids (NSC-153175 and NSC-153174), both stereoisomers of which are highly active. As in the L1210 system, the activity of the nitrosoureas against Lewis lung carcinoma (log 1/C), at least against the early form of the disease, shows a parabolic relationship of the log of the partition coefficients of these compounds (9), but here also there is no separation of activity from toxicity.

Although the mechanism of action of the nitrosoureas has not been established (10,11), a number of studies on the behavior of these agents both in vitro and in vivo (10,11) indicate that certain chemically reactive compounds are produced under in vivo conditions (12,13). The normal decomposition of an *N,N'*-disubstituted nitrosourea in water results in the production of an alcohol derived from the group attached to the nitrosated nitrogen, nitrogen, carbon dioxide, and a symmetric urea, both substituents of which derive from the group attached to the unnitrosated urea nitrogen (fig 6) (12). *N*-(2-fluoroethyl)-*N*-nitrosoureas decompose in the normal manner, but because of the lability of the carbon-chlorine bond, the decomposition of the *N*-(2-chloroethyl)-*N*-nitrosoureas is more complicated (fig 7) (12–16). In distilled water the products of decomposition are HCl, nitrogen, acetaldehyde, and a primary amine derived from the group attached to the unnitrosated nitrogen (the HCl produced forms the amine HCl, preventing urea formation) (13). In phosphate buffer at pH 7.4 (an approximation of physiologic conditions), about half of the nitrosourea decomposes normally to give 2-chloroethanol and half abnormally to give acetaldehyde (12–14,16), which is at least partly produced by pathway *a* and may also be produced by pathway *b* (fig 7), although the decomposition of 5-[3-(2-chloroethyl)-1-triazenyl]-imidazole-4-carboxamide in water, which must proceed via a 2-chloroethyl carbocation, gives 2-chloroethanol and no acetaldehyde (17). Regardless of the mechanistic details of these decompositions, it is clear that a number of reactive species (fig 8) are produced. The reaction of one or more of these reactive species with biologic macromolecules is almost certainly responsible for both the anticancer activity and toxicity of the nitrosoureas. Although MNU is known to react with DNA (to a much lesser extent than mechlorethamine, however) (18–22), the lack of anti-DNA-viral activity of three (2-chloroethyl)nitrosoureas (table 9) compared to the activity of mechlorethamine and BCNU (which produces 2-chloroethylamine, a mustard type) raises a question as to the biologic significance of the reaction with DNA. At the same time, it has not been possible to relate the carbamoylation of proteins (by the isocyanate generated in vivo [fig 9]) with biologic activity (10,11).

Table 6. —The effect of modification of the 2-chloroethyl group on Leukemia L1210 activity (10^5 Cells, IP)

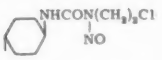
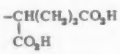
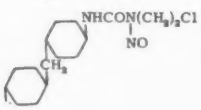
R_x: IP, single dose

	RN(NO)CONHR'		Optimal dose,	
NSC No.	R	R'	mg/kg	% ILS *
95465	$ClCHCH_2$ | Me	CH_2CHCl | Me	1000	140 †
			750	97
95460	$ClCHCH_2$ | Me		750	87
81701	$Cl(CH_2)_3$			inactive
95462	$ClCH_2CH-$ | Et	$-CHCH_2Cl$ | Et		inactive
87426	Cl	Cl		inactive
102864				inactive

* Percent increase in lifespan.

† 1 survivor/6.

Table 7.—Nitrosoureas Highly Active Against Leukemia L1210 (10^5 Cells, IP)
R_x: single dose, IP

NSC No.	X	$X(CH_2)_2N(NO)CONHR$ R	Cytotoxicity Index,* μM	LD_{10}, mmol/kg	ED_{50},† mmol/kg	LD_{10}/ED_{50}	Log $1/C^{\ddagger}$	Log P
136902	Cl		30	0.14	0.026	5	1.6	-0.90
93372	Cl		100	0.72	0.19	4	0.73	2.7
128303	Cl		100	0.085	0.023	4	1.6	2.1
155950	Cl		270	0.19	0.051	4	1.8	2.6
175377	Cl		80	0.14	0.041	3	1.4	-0.64
132085	F		40	0.16	0.047	3	1.3	1.54
129967	Cl		100	0.36	0.12	3	0.93	3.83
106767	F		40	0.12	0.037	3	1.4	-0.41
90330	Cl		300	0.61	0.19	3	0.72	2.19
93184	F (Cl)	$(CH_2)_2Cl$ (F)	40	0.32	0.056	3	1.3	0.93
207115	Cl		110	0.16	0.055	3	1.3	-0.40
163478	Cl		180	0.92	0.34	3	0.47	-2.2
136900	F		120	0.24	0.088	3	1.05	0.37
143147			50	0.52	0.20	3	0.70	3.1
93170	Cl		180	0.48	0.18	3	0.74	3.3
204818	Cl		90	0.12	0.048	3	1.3	2.6
239717	Cl		40	0.14	0.056	3	1.3	1.0
117624	Cl		60	0.15	0.062	3	1.2	2.6
95441	Cl		120	0.17	0.073	2	1.1	3.3
79037	Cl		40	0.24	0.12	2	0.80	2.8
409962	Cl	CH_2CH_2Cl	14	0.19	0.09	2	1.0	1.5

* Dose required to inhibit the growth of treated KB cells to 50% of controls. † Dose required for 50% cures. ‡ $C = ED_{50}$.

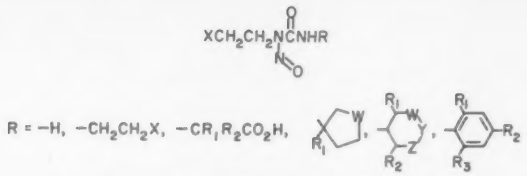

XCH2CH2NCNHR
NO
R = −H, −CH2CH2X, −CR1R2CO2H,
X = halogen; W, Y, and Z = carbon, substituted carbon, or hetero atom; R1, R2, and R3 = alkyl or other substituent.
R =
or
No cures at the LD10.

FIGURE 2.—Nitrosoureas highly active against L1210 leukemia (≥ 50% cures).

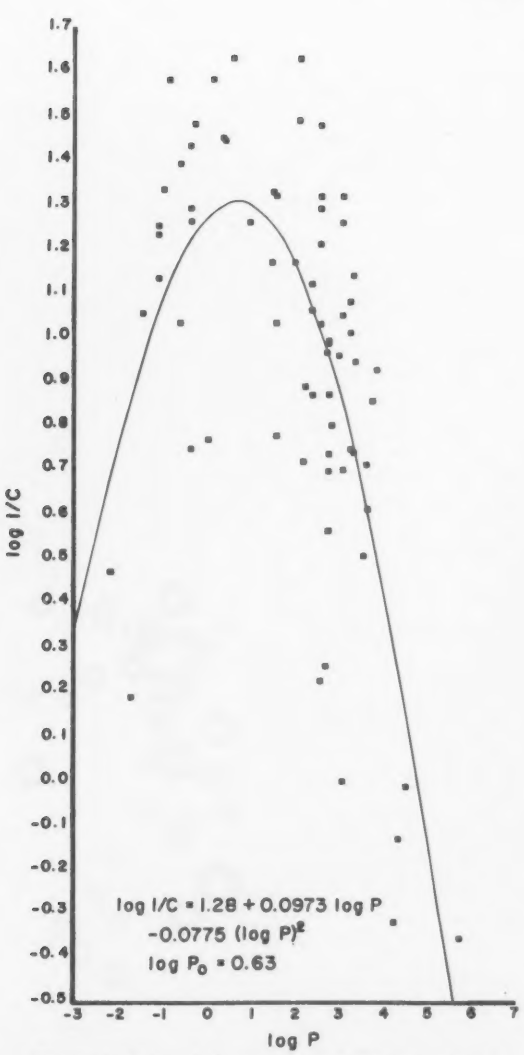


FIGURE 3.—Selected nitrosoureas vs L1210 leukemia (ip).

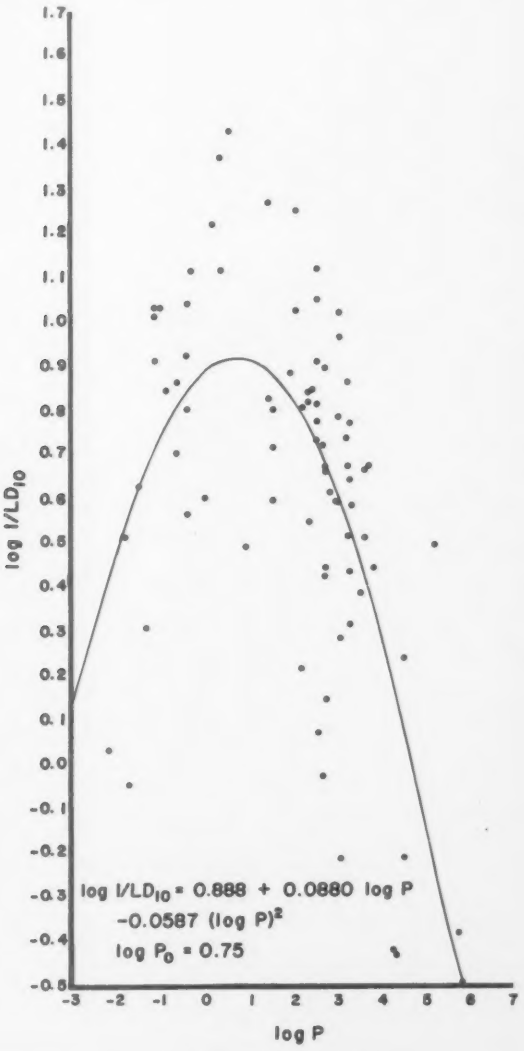


FIGURE 4.—Toxicity of selected nitrosoureas.

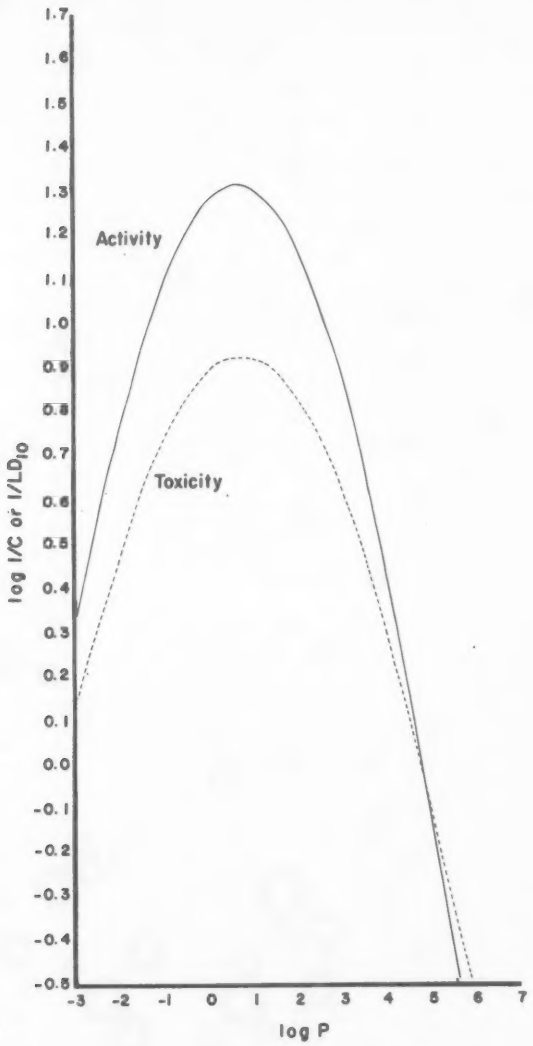
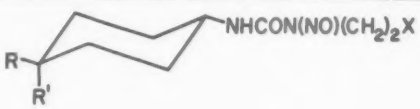


FIGURE 5.—Activity and toxicity of selected nitrosoureas.

Recently May et al (23,24), Reed and May (25), Hill et al (26), and Hilton and Walker (27) have observed the rapid hydroxylation of CCNU by liver microsomes from rodents and by humans treated with this drug. The data indicate that the rate of metabolic hydroxylation exceeds the rate of chemical breakdown of CCNU, and therefore, it is likely that the hydroxylated metabolites are immediate precursors of the therapeutically active moieties. Five of the six possible isomeric monohydroxy derivatives of CCNU (fig 10) were identified by Reed and Hilton. All six of these compounds have been synthetically prepared (28)[4] and their chemical and biologic properties (11) compared with those of CCNU (table 10). Although some variation in half-life ($T_{0.5}$) and alkylating ability (A_{540}) was observed, the most outstanding difference in these compounds is their carbamoylating ability (DPM), with the *cis*- and *trans*-2-hydroxy and the *cis*-3-hydroxy compounds showing

[4]Johnston TP, McCaleb GS, and Montgomery JA. A comparison of the chemical and biologic properties of the isomeric *N*-(2-chloroethyl)-*N'*-(hydroxycyclohexyl)-*N*-nitrosoureas. To be submitted to the *J Med Chem.*

Table 8.--Nitrosoureas vs advanced Lewis lung carcinoma: single dose given ip on Day 7



Highly Active				Inactive			
NSC No.	R	R'	X	NSC No.	R	R'	X
95441	Me	H	Cl	135091	H	Me	Cl
93372	$Cl(CH_2)_2N(NO)CONH$	H	Cl	143667	H	$Cl(CH_2)_2N(NO)CONH$	Cl
153175	HO_2C	H	Cl	95459	$F(CH_2)_2N(NO)CONH$	H	F
224092	HO_2C	H	F	224094	H	HO_2C	F
103548	EtO_2C	H	Cl	239717	HO	H	Cl
153174	H	HO_2C	Cl	239724	H	OH	Cl
				79037	H	H	Cl

almost no carbamoylating activity, a property they have in common with chlorozotocin (NSC-178248) (29) (fig 11). Figure 11 shows the intramolecular carbamoylation reaction of both chlorozotocin and streptozotocin (NSC-85998), which presumably explains the low intermolecular carbamoylating activity of these compounds and of their hydroxycyclohexyl congeners previously referred to. Streptozotocin and chlorozotocin are unusual nitrosoureas of particular interest because they exhibit low bone marrow toxicity (30,31), a finding that may be related to their low carbamoylating activity. This lack of myelosuppressive activity plus its high-level activity against L1210 leukemia makes chlorozotocin a prime candidate for clinical trials.

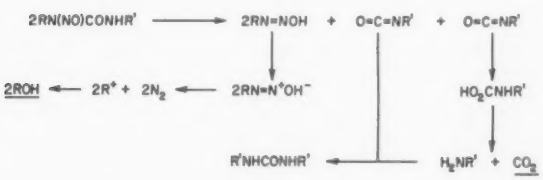


FIGURE 6.—Normal decomposition of *N*, *N'*-disubstituted nitrosoureas.

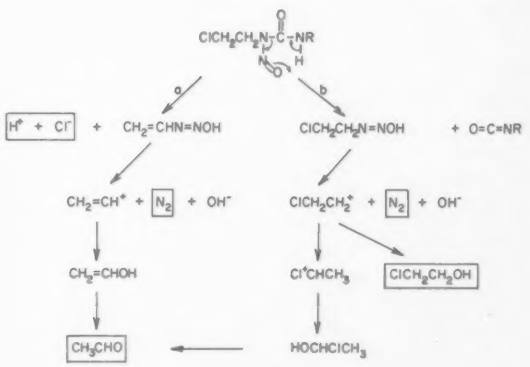


FIGURE 7.—Aqueous decomposition of *N*-(2-chloroethyl)-*N*-nitrosoureas.

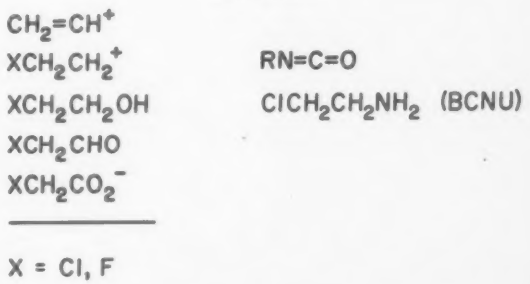


FIGURE 8.—Possible biologically active moieties resulting from the decomposition and metabolism of the nitrosoureas.

Table 9.—Antiviral Activity

Compound	Virus Rating			
	DNA		RNA	
	Vaccinia	Herpes simplex	Rhino 1A	Influenza PR8
HN2	2.0	-	-	-
$ClCH_2CH_2CNU$ (BCNU)	1.9	-	-	-
	0.2	0.5	0	0
	0	0	0	0
$HO_2CCH_2CH_2(HO_2C)CHCNU$	0.2	0.1	0	0

CNU = $NHCON(NO)CH_2CH_2Cl$

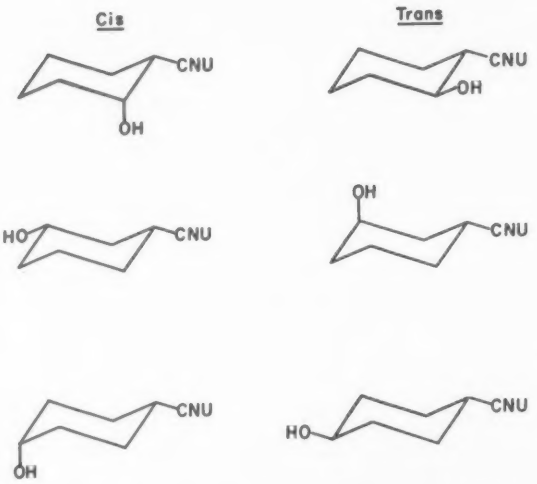


FIGURE 10.—Possible ring-hydroxylated metabolites of CCNU.

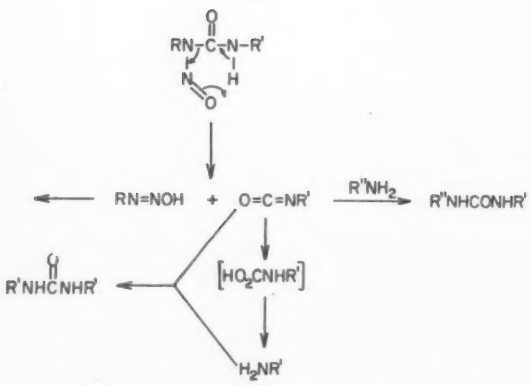


FIGURE 9.—Fate of the isocyanate moiety.

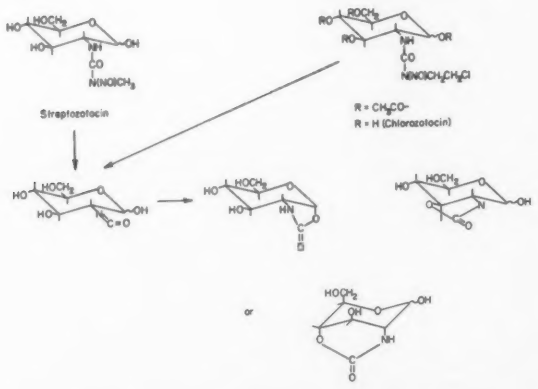


FIGURE 11.—Intramolecular carbamoylation reaction.

Table 10. —Properties of Metabolites of CCNU

Compound	Percent of value for CCNU					ED_{50}/LD_{10}
	$T_{0.5}$	A_{540}	DPM	LD_{10} (mmoles/kg)	ED_{50} (mmoles/kg)	
CCNU	100	100	100	100	100	0.65
Chlorozotocin	46	489	4	38	30	0.52
cis-2-OH	76	177	1	80	56	0.44
trans-2-OH	66	329	6	34	16	0.30
cis-3-OH	91	133	21	80	47	0.37
trans-3-OH	84	112	61	70	43	0.40
cis-4-OH	93	136	104	55	55	0.65
trans-4-OH	87	140	100	58	35	0.40

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Nitrosoureas: A Review of Experimental Antitumor Activity[1]

F. M. Schabel, Jr[2,*]

SUMMARY

The chemical class of drugs known as the nitrosoureas are a recently developed group of very active alkylating-agent anticancer drugs which are best represented by BCNU, CCNU, and methyl-CCNU (meCCNU). The nitrosoureas are among the most active, if not the most active, anticancer drugs both quantitatively (log kill of sensitive tumor cells in vivo) and qualitatively (spectrum of mouse, rat, and hamster tumors responding to treatment). Therapeutic anticancer activity of the nitrosoureas has been consistently observed with oral as well as parenteral administration. The nitrosoureas are clearly the most active group of anticancer drugs observed against experimental meningeal leukemias and intracerebrally implanted transplantable primary tumors of central nervous system origin (eg, gliomas, ependymoblastomas, and astrocytomas in mice and hamsters). The nitrosoureas have been observed to be less than additive in lethal toxicity for vital normal cells in the mouse in combination with representatives of the other major classes of anticancer agents, eg, purine antagonists, pyrimidine antagonists, inhibitors of DNA polymerase(s) or ribonucleotide reductase(s), mitotic inhibitors, drugs that bind to or intercalate with DNA, and other alkylating agents. Therapeutic synergism against one or more transplantable or spontaneous tumors of mice, rats, or hamsters with one of several nitrosoureas in two-drug combinations with representatives of most of the major classes of anticancer agents listed above has been reported. With a number of advanced-staged mouse tumors, generally considered to be refractory to treatment with most anticancer agents, long-term cures have been obtained with combination-drug or combined-modality (surgery plus chemotherapy) treatment. The demonstrated lack of cross-resistance of several leukemias and solid tumors of mice selected for resistance to BCNU, meCCNU, or other alkylating agents suggests that the widely held opinion that all alkylating agents are very similar in biologic mechanism of action, and therefore resistance to one alkylating agent probably predicts cross-resistance to all alkylating agents, may no longer be tenable. If not, then alkylating-agent drug combinations, either used alone or combined with other treatment modalities (eg, surgery) which have been reported to result in therapeutic improvement in a number of experimental murine tumor systems, may be indicated for serious consideration as surgical adjuvant chemotherapy by surgeons or as primary therapy by medical oncologists.

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Limited but reproducible therapeutic activity of 1-methyl-3-nitro-1-nitrosoguanidine (MNNG) (fig 1) against L1210 leukemia in mice was first detected in 1959 in the Cancer Chemotherapy National Service Center screening program. We examined other compounds structurally related to MNNG and observed that 1-methyl-1-nitrosourea (MNU) (fig 1) was more effective than MNNG in mice implanted ip with L1210 cells. In addition, and in contrast to methotrexate, 6-mercaptopurine, 5-fluorouracil (5-FU), azaserine, cyclophosphamide (CPA), mitomycin C, and MNNG, MNU given ip had limited but reproducible therapeutic activity in mice implanted intracerebrally (ic) with L1210 cells (1,2). These observations stimulated great interest in the nitrosoureas because (*a*) these drugs represented a new chemical class of anticancer agents, and (*b*) they suggested that the problem of the so-called pharmacologic sanctuaries[4] against otherwise effective anticancer drugs might be solved.

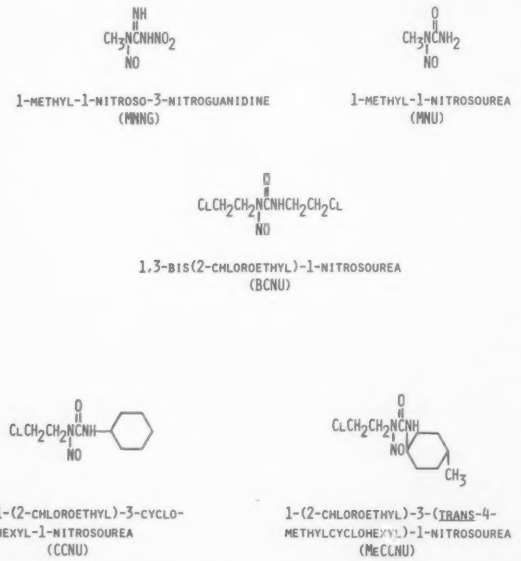


FIGURE 1.—Structures of MNNG, MNU, BCNU, CCNU, and meCCNU.

For these reasons, an active program of congener synthesis of nitrosoureas was begun at the Southern Research Institute. The 23rd nitrosourea synthesized in this program was BCNU (fig 1). Early studies showed BCNU to be curative[5] in some mice implanted with $\leq 10^7$ L1210 cells ip, 10^6 L1210 cells iv, or 10^6 L1210 cells ic with single doses ($\leq$ LD10)[6] given either ip or orally[7] (2,3) (table 1). BCNU was observed to be more active at equitoxic ($\leq$ LD10) doses against ip, iv, or ic implanted L1210 than was CPA. Representative data from an early experiment comparing BCNU and CPA are shown in table 2. They indicated that, based on cure rates, CPA was about one tenth as active as BCNU against ip or iv implanted L1210 cells and was essentially inactive against ic implanted L1210 cells (3).

BCNU was also much less dependent on the treatment schedule for optimum therapeutic activity against L1210 than was CPA (3). The data in table 3 clearly indicate that the optimal treatment schedule for CPA against ip implanted L1210 was single-dose treatment which became progressively less active on multiple-dose treatment as the interval between doses was shortened. BCNU had marked activity (curative, except on repeated daily treatment) on all treatment schedules shown against these large L1210 tumor cell populations. The data in table 3 also clearly indicate the requirement for internal tovicity control treatment groups for objective comparative evaluation of therapeutic activity, particularly with murine leukemias with which the increase in life-span (ILS) of treated mice over that of untreated control mice is commonly used as the therapeutic activity end point (eg, the greatest observed ILS after receiving a single dose of CPA was at the LD40 level, and on the qd Days 2–16 or the q2d Days 2–16 schedule it was at the LD100 level) (table 3). Comparing the ILS at lethal drug doses is unrealistic and must be recognized and considered in making objective comparisons of antitumor activity between anticancer agents.

THERAPEUTIC COMPARISONS WITH CONGENERS OF BCNU

The marked and readily reproducible activity of BCNU against ip, iv, or ic implanted L1210 cells provided indication for additional congener syntheses. In determining therapeutic activity of new structures, and for the reasons cited above, special emphasis was placed on the therapeutic activity against ic implanted L1210 cells. Initial

[4]*Pharmacologic sanctuaries:* Many anticancer drugs do not appear in some organs or tissues, and especially the central nervous system (CNS), in lethal concentrations for the contained tumor cells with drug doses at or below limiting toxicity for vital normal cells. The pharmacologic sanctuary of the CNS is particularly critical in (*a*) meningeal leukemia, (*b*) primary tumors of the CNS, and (*c*) metastasis to the CNS from other primary sites.

[5]*Curative:* Able to destroy a large enough fraction of a viable tumor cell population so that the cells surviving treatment can never re-establish the grossly apparent disease.

[6]Single-dose LD10 of BCNU in BDF$_1$ mice is the same (about 41 mg/kg), and the therapeutic activity is essentially equal against L1210 when given either ip or orally.

[7]Drugs given orally were delivered into the stomach by gastric intubation.

Table 1

Early Observations on the Therapeutic Activity of BCNU vs L1210

L1210 Cell Implant		BCNU, Single-Dose LD_{10} (41 mg/kg), Day 2 Postimplant			
		I.P.		P.O.	
Size	Site	Median ILS (%)*	45- to 60-Day Survivors/Total	Median ILS (%)*	45- to 60-Day Survivors/Total
10^7	i.p.	+350	6/10	+333	3/10
10^6		+340	9/10	+227	2/10
10^5		+ 33	9/10	+122	8/10
10^7	i.v.	+300	0/10	+337	0/10
10^6		+250	2/10	+283	7/10
10^5		+257	7/10	-	10/10
10^6	i.c.	+ 16	0/10	+250	4/10
10^5		+126	4/10	+271	7/10
10^4		- 23	7/10	+ 33	7/10

*% ILS = Median life span of treated mice greater than median life span of untreated control mice in percent, excluding survivors.

studies with CCNU (fig 1) suggested it to be more effective than BCNU against ic implanted L1210 cells. CCNU cured about 80% and BCNU < 50% of the mice implanted ic with 10^4 L1210 cells. CCNU cured some mice implanted ic with 10^6 L1210 cells, while BCNU failed to cure such mice (5). Based on its apparent greater activity against experimental meningeal L1210 leukemia, we selected CCNU for more detailed laboratory study.

About 80% of the more than 250 nitrosoureas that we have examined for activity against ip implanted L1210 have met or exceeded minimal therapeutic response (≥ 25% ILS) for activity in this primary screening system on single-dose treatment. Ninety-nine of these compounds have cured ≥ 50% of the mice bearing ≥ 10^5 L1210 cells at the time of single-dose treatment with ≤ LD10 doses. In the absence of other compelling factors, this degree of curative activity in the L1210 system is currently the basis for consideration of new nitrosoureas for additional secondary therapy trials in L1210 and/or other tumor systems.

In 1969, we began evaluating these most active nitrosoureas against established[a] transplanted Lewis lung (LL) carcinoma. LL carcinoma was chosen because it had been shown to be consistently responsive to treatment with CPA (4).

The marked activity of methyl-CCNU (meCCNU) (fig 1) against the established LL carcinoma was observed soon after comparative studies of these most active nitrosoureas were begun, and it is still among the most active of the nitrosoureas against the LL tumor.

[a] *Established:* Sc implanted LL tumor with treatment started ≥ 7 days after implant, when the primary tumors are about 400 (±50) mg, and at which time most have spontaneously metastasized to the lungs.

Table 2

Therapeutic Activity of BCNU and CPA versus L1210 Leukemia

L1210 Cell Implant Size	Site	BCNU, Single-Dose LD_{10}, Day 2 IP: Median ILS (%)*	BCNU, Single-Dose LD_{10}, Day 2 IP: 45- to 60-Day Survivors/Total	CPA, Single-Dose LD_{10}, Day 2 IP: Median ILS (%)*	CPA, Single-Dose LD_{10}, Day 2 IP: 45- to 60-Day Survivors/Total
10^7	IP	+350	6/10	+180	0/10
10^6		+340	9/10	+142	4/10
10^5		+ 33	9/10	+117	2/10
10^4				+300	9/10
10^7	IV	+300	0/10	+275	0/10
10^6		+250	2/10	+166	0/10
10^5		+257	7/10	+100	7/10
10^4				+100	9/10
10^6	IC	+ 16	0/10		
10^5		+126	4/10		
10^4		- 23	7/10	- 11	0/10
10^3				+ 2	0/10
10^2				+ 1	0/10

*% ILS = Median life span of treated mice greater than median life span of untreated control mice in percent, excluding survivors.

For the reasons briefly mentioned above, the most extensive in vivo therapy studies carried out by the Southern Research Institute and many other laboratories around the world have been with BCNU, CCNU, and meCCNU. Studies on these compounds through 1972 have been summarized in review-type reports (3,5). Since BCNU, CCNU, and meCCNU have been the most widely studied and are among the most unique of the nitrosoureas from the standpoint of recognized biologic activity, they will be the primary subjects of my discussions to follow.

COMPARATIVE THERAPEUTIC ACTIVITY OF BCNU, CCNU, AND MECCNU AGAINST L1210 LEUKEMIA AND LL CARCINOMA

L1210 Leukemia

In table 4 are listed representative data from a large total experience with BDF_1 mice implanted ip, iv, or ic with L1210 cells and treated ip or orally with BCNU, CCNU, or meCCNU. Several interesting and probably important characteristics of these

TABLE 3

Comparison of the Activity of BCNU and Cyclophosphamide (CPA) on Various Treatment Schedules in Mice Implanted IP, with 10^7 L1210 Cells

Treatment Schedule[1] (Days Post Implant of L1210 Cells)	BCNU			CPA		
	Dose (mg/kg/dose)	% ILS[2] (Survivors)	Normal Mouse Toxicity Survivors/ Total	Dose (mg/kg/dose)	% ILS[2] (Survivors)	Normal Mouse Toxicity Survivors/ Total
Day 2 only	88	16	0/10	350	400 (1/10)	6/10
	59	83 (4/10)	2/10	300	340	8/10
	39*	283 (5/10)*	9/10*	250*	240*	10/10*
	26	258 (4/10)	10/10	200	210	10/10
	17	150 (1/10)	10/10	150	160	10/10
qd 2-16 days	7.3	308 (4/10)	3/10	90	240	0/10
	4.9*	91 (1/10)*	9/10*	60	160	2/10
	3.2	50	10/10	40	90	8/10
	2.1	16	10/10	27*	30*	10/10*
q2dx8, Days 2-16	22	216	0/10	225	120	0/5
	15	233 (4/10)	8/10	100	340	0/10
	9.8*	316 (5/10)*	10/10*	67	190	2/10
	6.4	125	10/10	44*	60*	10/10*
	4.2	33	10/10	30	20	10/10
q4dx4, Days 2,6,10,14	44	116	0/10	270	190	0/10
	29	141 (6/10)	3/10	180	350	1/10
	20*	383 (6/10)*	9/10*	120	320	8/10
	13	300 (2/10)	10/10	80*	140*	10/10*
	8.4	133	10/10	54	40	10/10
q3hx8, Days 2,6,10,14	4.4	208	0/10	45	300	0/10
	2.9*	416*	10/10*	30	280	4/10
	2.0	483 (3/10)	9/10	20	200	6/10
	1.3	250	10/10	13	90	4/10
	0.84	66	9/9	8*	40*	10/10*

*Highest nontoxic dose — $\leq LD_{10}$ in normal (nonleukemic) mice.
[1]All therapy IP and begun 48 hours postimplant of L1210 cells.
[2]% ILS = Median life span of treated mice greater than median life span of untreated control mice in percent, excluding 45-day survivors. Numbers in parentheses are 45-day survivors/total in treated groups.

very active nitrosoureas are quickly apparent from these data:

(*a*) BCNU, CCNU, and meCCNU cure some mice when given ip or orally following implant of $\leq 10^7$ L1210 cells ip or iv and following implant of $\leq 10^6$ L1210 cells ic. Against ic implanted L1210, CCNU is more active than BCNU, which in turn is more active than meCCNU. However, it should be emphasized that the differences in therapeutic activity between BCNU, CCNU, and meCCNU against ip, iv, or ic implanted L1210 are seldom greater than one order of magnitude (tenfold) of tumor cell kill at equitoxic doses, which is probably insufficient to make reliable selections among active nitrosoureas for further studies in other tumor systems.

(*b*) BCNU, CCNU, and meCCNU have very similar toxicities for vital normal cells (lethal toxicity) and are similar in therapeutic activity against ip, iv, or ic implanted L1210 cells when given either ip or orally. This is particularly interesting considering

Table 4

Comparative Therapeutic Activity of BCNU, CCNU, and MeCCNU against L1210

BCNU

(Single-Dose LD_{10}: IP = 41 mg/kg; PO = 41 mg/kg)

L1210 Cell Implant Route	L1210 Cell Implant Size	Treatment: Day 1 or 2 Postimplant Route	Single Dose (mg/kg/dose)	Fraction of LD_{10}	Median Life Span (Days); (Dying Animals Only) Control	Treated	% ILS	45- to 60-Day Survivors[1] (%)	Toxicity Control[2] Survivors/ Total
IP	10^7	IP	54-45	1.3-1.1	6.0	9.5	+ 58	28/70	36/70
			39-30	1.0-0.7	6.0	19.2	+220	130/229 (57)	176/189
			27-16	0.7-0.4	6.0	19.0	+216	79/288 (27)	204/209
		PO	59	1.4	6.0	14.0	+133	0/50 (0)	3/10
			40	1.0	6.0	15.0	+150	6/60 (10)	10/10
			20	0.5	6.0	16.5	+175	3/60 (5)	20/20
IP	10^6	IP	50-45	1.2-1.1	7.5	13.0	+ 73	8/20	8/20
			39-30	1.0-0.7	7.5	25.5	+240	109/150 (73)	110/118
			27-16	0.7-0.4	7.5	18.8	+150	47/130 (36)	80/80
		PO	40	1.0	9.0	20.5	+127	4/10 (40)	-
			20-15	0.5-0.4	9.0	17.0	+ 88	22/119 (18)	20/20
IP	10^5	IP	50	1.2	9.0	9.0	0	7/10	4/10
			40-30	1.0-0.7	9.0	16.7	+ 85	174/220 (79)	163/166
			25-16	0.6-0.4	9.0	18.9	+110	188/302 (62)	158/161
		PO	40-30	1.0-0.7	9.0	21.8	+142	14/20 (70)	-
			20-15	0.5-0.4	9.0	17.7	+ 96	21/50 (42)	-
IV	10^7	IP	40-30	1.0-0.7	4.0	14.5	+262	3/200 (2)	182/187
			26-16	0.6-0.4	4.0	12.8	+220	1/209 (<1)	187/188
		PO	40	1.0	4.0	18.5	+362	2/20 (10)	10/10
			24-20	0.6-0.5	4.0	16.3	+307	0/10 (0)	-
IV	10^6	IP	40-30	1.0-0.7	5.0	17.3	+246	8/69 (12)	56/60
			24-16	0.6-0.4	5.0	13.9	+178	3/90 (3)	48/50
		PO	40	1.0	5.8	25.0	+331	13/20 (65)	10/10
			27-20	0.7-0.5	5.8	19.8	+241	2/20 (10)	10/10
IV	10^5	IP	40-30	1.0	7.0	20.0	+185	27/70 (39)	50/50
			24-16	0.6-0.4	7.0	16.9	+141	5/90 (6)	40/40
		PO	40	1.0	7.0	-	-	10/10 (100)	-
			20	0.5	7.0	16.5	+135	0/10 (0)	-
IC	10^6	IP	40-31	1.0	6.0	13.5	+125	4/30 (13)	26/29
			25-16	0.7-0.4	6.0	12.4	+106	0/40 (0)	30/30
		PO	40	1.0	6.0	20.0	+233	5/10 (50)	-
			20	0.5	6.0	14.5	+141	0/40 (0)	-
IC	10^5	IP	39	1.0	7.8	17.0	+117	4/10 (4)	8/10
			25-16	0.6-0.4	7.8	13.8	+ 76	2/30 (7)	10/10
		PO	40	1.0	7.0	23.5	+235	1/10 (10)	-
			24-20	0.6-0.5	7.0	16.0	+128	2/20 (10)	10/10
IC	10^4	IP	40-30	1.0	9.0	15.1	+ 67	37/70 (53)	55/60
			25-15	0.6-0.4	9.0	16.3	+ 81	15/69 (22)	60/60
		PO	40	1.0	9.0	12.0	+ 33	7/10 (70)	-
			20	0.5	9.0	15.5	+ 72	2/10 (20)	-

[1] Survivors in drug-treated/total. No survivors in untreated leukemia-control groups.

[2] 30-Day survivors/total in parallel group of normal (nonleukemic) mice treated with the same doses of drug.

(continued)

Table 4 (continued)

CCNU

(Single-Dose LD_{10}: IP = 48 mg/kg; PO = 46 mg/kg)

L1210 Cell Implant		Treatment: Day 1 or 2 Postimplant			Median Life Span (Days); (Dying Animals Only)			45- to 60-Day	Toxicity Control[2]
Route	Size	Route	Single Dose (mg/kg/dose)	Fraction of LD_{10}	Control	Treated	% ILS	Survivors[1] (%)	Survivors/ Total
IP	10^7	IP	50-40	1.0-0.8	6.0	21.3	+255	92/129 (71)	77/80
			32-22	0.7-0.5	6.0	19.0	+216	34/144 (24)	99/100
		PO	24	0.5	6.0	14.0	+133	1/20 (5)	10/10
IP	10^6	IP	50	1.0	7.0	17.0	+142	66/90 (73)	27/30
			32-25	0.7-0.5	7.0	16.0	+128	9/40 (23)	19/19
		PO	24	0.5	8.0	17.0	+112	0/10 (0)	-
IP	10^5	IP	50-40	1.0-0.8	9.0	18.0	+100	135/166 (81)	87/90
			34-22	0.7-0.5	9.0	17.4	+ 93	46/116 (40)	77/79
		PO	50	1.1	9.3	-	-	20/20 (100)	10/10
			24	0.5	9.3	16.0	+ 72	4/10 (40)	-
IV	10^7	IP	50-40	1.0-0.8	4.0	16.3	+307	60/244 (25)	232/249
			38-22	0.8-0.5	4.0	12.8	+220	16/335 (5)	286/290
		PO	49	1.1	4.0	24.0	+500	6/10 (60)	-
			24	0.5	4.0	15.0	+275	0/10 (0)	-
IV	10^6	IP	50-40	1.0-0.8	6.0	17.5	+191	36/70 (51)	56/59
			27-25	0.6-0.5	6.0	13.0	+116	8/50 (16)	29/30
		PO	24	0.5	6.0	13.0	+116	0/10 (0)	-
IV	10^5	IP	50-45	1.0-0.9	7.0	23.5	+235	24/30 (80)	18/19
			38-25	0.8-0.5	7.0	14.5	+107	18/50 (36)	19/20
		PO	24	0.5	7.0	16.0	+128	8/10 (80)	-
IC	10^6	IP	50-45	1.0-0.9	6.0	14.8	+146	13/20 (65)	17/18
			25-21	0.5-0.4	6.0	15.0	+150	1/20 (5)	10/10
		PO	49	1.1	6.0	9.0	+ 50	7/10 (70)	-
			24	0.5	6.0	16.0	+166	0/10 (0)	-
IC	10^5	IP	50-45	1.0-0.9	7.8	16.2	+107	14/30 (47)	18/20
			33-25	0.7-0.5	7.8	15.5	+ 98	5/30 (17)	10/10
		PO	50	1.1	8.3	10.5	+ 26	14/20 (70)	10/10
			24	0.5	8.3	17.0	+104	5/10 (50)	-
IC	10^4	IP	50-40	1.0-0.8	9.0	18.3	+103	107/150 (71)	72/80
			38-25	0.8-0.5	9.0	14.1	+ 56	54/170 (32)	90/100
		PO	50	1.1	9.0	21.0	+133	24/30 (80)	20/20
			25	0.5	9.0	17.3	+ 92	8/20 (40)	10/10

[1] Survivors in drug-treated/total. No survivors in untreated leukemia-control groups.

[2] 30-Day survivors/total in parallel group of normal (nonleukemic) mice treated with the same doses of drug.

that these compounds are highly reactive and of limited chemical stability, and probably act biologically as alkylating agents. The oral activity against ic implanted tumor cells is quite remarkable considering that with oral treatment the drug must be absorbed from the stomach or gut (at either high or low pH, depending on the site of absorption), be carried by the blood, and then cross the blood-brain barrier in sufficient quantity to retain significant cytotoxic activity against L1210 cells in the CNS.

LL Carcinoma

As indicated above, the superior activity of me-CCNU against established LL carcinoma over that of other nitrosoureas with marked activity against

Table 4 (continued)

MeCCNU

(Single-Dose LD_{10}: IP = 42 mg/kg; PO = 42 mg/kg)

L1210 Cell Implant Route	L1210 Cell Implant Size	Treatment: Day 1 or 2 Postimplant Route	Single Dose (mg/kg/dose)	Fraction of LD_{10}	Median Life Span (Days); (Dying Animals Only) Control	Treated	% ILS	45- to 60-Day Survivors[1]	(%)	Toxicity Control[2] Survivors/ Total
IP	10^7	IP	36-24	0.9-0.6	6.0	23.3	+288	34/60	(57)	60/60
			16-10	0.4-0.2	6.0	11.8	+ 96	9/40	(23)	20/20
		PO	36-24	0.9-0.6	6.0	17.0	+183	7/20	(35)	20/20
IP	10^6	IP	36-24	0.9-0.6	8.0	19.8	+147	28/40	(70)	40/40
			16-11	0.4-0.3	8.0	14.0	+ 75	1/20	(5)	-
		PO	36-24	0.9-0.6	8.0	18.5	+131	14/20	(70)	20/20
IP	10^5	IP	36-24	0.9-0.6	8.5	16.8	+ 97	54/70	(77)	40/40
			18-13	0.4-0.3	8.5	14.2	+ 67	9/40	(18)	30/30
		PO	36-24	0.9-0.6	9.0	25.0	+177	18/20	(90)	20/20
IV	10^7	IP	36-24	0.9-0.6	4.0	17.2	+330	61/245	(25)	250/250
			20-12	0.5-0.3	4.0	11.4	+185	4/178	(2)	148/149
		PO	36-24	0.9-0.6	4.0	17.3	+332	5/60	(8)	57/59
			16-12	0.4-0.3	4.0	9.0	+125	0/20	(0)	14/15
IV	10^6	IP	36-24	0.9-0.6	5.5	18.6	+238	70/160	(44)	157/159
			20-12	0.5-0.3	5.5	11.3	+105	2/100	(2)	78/79
		PO	36-24	0.9-0.6	5.5	18.3	+232	24/50	(48)	48/49
			16	0.4	5.5	11.0	+100	0/10	(0)	4/5
IV	10^5	IP	36-24	0.9-0.6	7.0	25.2	+260	108/140	(77)	140/140
			20-12	0.5-0.3	7.0	13.8	+ 97	14/110	(13)	89/100
		PO	36-24	0.9-0.6	7.0	20.2	+188	39/60	(65)	57/59
			16-12	0.4-0.3	7.0	12.5	+ 78	0/20	(0)	14/15
IC	10^6	IP	36-24	0.9-0.6	6.0	14.2	+136	2/90	(2)	89/90
			13	0.3	6.0	8.5	+ 41	0/70	(0)	70/70
		PO	36-24	0.9-0.6	6.0	13.2	+120	0/30	(0)	25/25
			12	0.3	6.0	8.0	+ 33	0/10	(0)	10/10
IC	10^5	IP	36-24	0.9-0.6	7.0	11.8	+ 68	0/20	(0)	20/20
		PO	36-24	0.9-0.6	7.0	12.8	+ 82	1/20	(5)	15/15
IC	10^4	IP	36-24	0.9-0.6	9.0	15.6	+ 73	18/120	(15)	89/90
			18-13	0.4-0.3	9.0	12.5	+ 38	1/90	(1)	70/70
		PO	36-24	0.9-0.6	9.0	15.8	+ 75	2/30	(7)	25/25
			12	0.3	9.0	11.0	+ 22	0/10	(0)	10/10

[1] Survivors in drug-treated/total. No survivors in untreated leukemia-control groups.

[2] 30-Day survivors/total in parallel group of normal (nonleukemic) mice treated with the same doses of drug.

L1210 leukemia was the basis for selecting it for in-depth experimental therapy trials. Data from a representative experiment, in which we compared meCCNU with CCNU and BCNU against established LL carcinoma, are shown in figure 2. Optimum therapeutic response of established LL carcinoma to equitoxic doses (≤ LD10) of BCNU, CCNU, or meCCNU using the previously determined optimal treatment schedule (two equal doses, 6–8 days apart), indicated marked superiority of meCCNU over CCNU or BCNU based on (*a*) duration of regression and regrowth of the primary tumor, and (*b*) complete regression and long-term cure rates. LL carcinoma is generally considered to be quite insensitive to drug treatment and complete regression of established tumors (≤ 400 mg) and long-term cure of some was an unanticipated, new, and unique experimental observation.

BROAD-SPECTRUM EXPERIMENTAL ANTITUMOR ACTIVITY OF BCNU, CCNU, AND MECCNU

Published reports of therapeutic activity of BCNU, CCNU, and meCCNU when used as single-agent treatment against a variety of experimental tumors are listed in tables 5–7.

BCNU

Since BCNU has been known as an active anticancer drug and has been available to investigators for a longer time than either CCNU or meCCNU, it has been studied more extensively. Many of the early studies reported from the Southern Research Institute have been repeatedly confirmed in other laboratories. In addition, the spectrum of experimental tumors with which some degree of therapeutic response has been observed is broader than with any other single anticancer drug reported in the scientific literature. The activity of BCNU against experimental meningeal L1210 leukemia (ic implanted) has been extended to a variety of other transplanted leukemias and lymphomas, as well as to a number of solid tumors (both primary CNS tumors and others which are known to or might metastasize to the CNS) implanted ic in mice, rats, and hamsters. The reports of therapeutic activity of BCNU cited in table 5 include a variety of tumors known to be carcinogen or virus induced (or carcinogen or virus associated) and representatives of most, if not all, of the major histologic types of malignant neoplasms originating in a number of inbred and outbred strains of mice, rats, and hamsters. From my point of view, all of the requirements for a really promising new chemical class of experimental anticancer agents are broadly satisfied by the reported antitumor activity of BCNU.

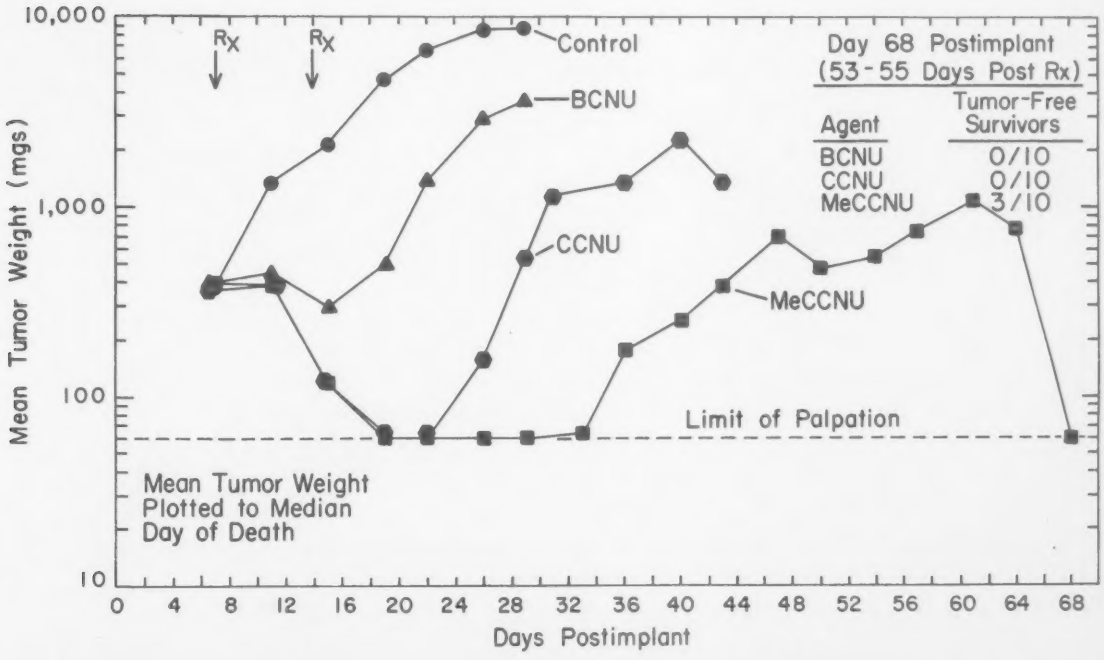


FIGURE 2.—Comparative activity of BCNU, CCNU, and meCCNU against established LL carcinoma. BDF_1 mice were implanted sc with approximately 20-mg tumor implants by trocar. Seven days after implant, when the tumors were about 400 mg (±50 mg) and had already metastasized to the lungs (4,61), treatment was started. Dose-response studies were done. The data plotted are mean tumor weights in groups of 10 mice, either untreated control or those treated with ≤ LD10s of BCNU, CCNU, or meCCNU on Day 7, and Day 13 (BCNU and meCCNU) or Day 15 (CCNU). Regression of all tumors in meCCNU-treated mice to below the level of palpation was observed. In 3 of these animals tumors did not recur.

Table 5

Published Reports of Therapeutic Activity of BCNU against Experimental Tumors

Tumor	Host	Implant[1] Site	Treatment[1] Route	References
A. Leukemias and Lymphomas				
1. Transplanted				
(a) Mouse				
L1210	Mouse	i.p.	i.p.	2,3,5,6,7,8,9,10, 11,12,13,14,15,16, 17,18,19,20,21,22, 23
"	"	i.p.	p.o.	2,3,6,8,11
"	"	i.p.	s.c.	2,24,25
"	"	i.v.	i.p.	3,5,6,7,8,9,26
"	"	i.v.	p.o.	3
"	"	s.c.	i.p.	27,28
"	"	s.c.	i.v.	28
"	"	s.c.	p.o.	28,29
"	"	s.c.	s.c.	11,28,29,30,31,32, 33,34,35,36
"	"	i.m.	i.p.	14
"	"	i.c.	i.p.	2,3,5,6,7,8,9,11, 27,37,38
"	"	i.c.	p.o.	2,3,6,8,11
"	"	i.c.	s.c.	2,31,33,35,39
L5178Y	"	i.c.	N.S.	40
P815	"	i.p.	i.p.	3
LSTRA	"	s.c.	s.c.	31,41,42,43
"	"	s.c.	N.S.	44,45
AKR	"	i.v.	i.p.	46,47,48
"	"	i.v.	N.S.	49
(b) Rat				
Dunning	Rat	i.p. or i.c.	i.p.	50
Myeloid[2]	Rat	i.p.	i.p.	51
Nova	Rat	s.c.	i.p.	52
2. Spontaneous				
(a) Mouse				
AKR	Mouse	Spont.	i.p.	3,53,54,55,56
"	"	Spont.	N.S.	57

Table 5 (continued)

Tumor	Host	Implant[1] Site	Treatment[1] Route	References
B. Solid Tumors				
1. Transplanted				
(a) Mouse				
C1025	Mouse	N.S.	i.p.	58
"	"	i.p.	i.p.	59
EO771	"	i.p.	i.p.	59
Ca755	"	s.c.	i.p.	3
EMT 6	"	s.c.	i.p.	60
Lewis lung	"	s.c.	i.p.	3,5,61,62
"	"	i.v.	i.p.	5,61
Lewis bladder	"	N.S.	i.p.	58
Colon 26	"	s.c.	i.p.	88
"	"	i.v.	i.v.	88
Colon 36	"	s.c.	i.v.	88
Taper liver	"	i.p.	i.p.	59
B16 melanoma	"	s.c.	i.p.	5, 96
"	"	i.m.	i.p.	67
"	"	i.p.	i.p.	59,63
"	"	i.c.	i.p.	63
C1300 neuroblastoma	"	s.c.	i.p.	64
Sarcoma 180	"	N.S.	i.p.	58
"	"	s.c.	i.p.	3
"	"	i.c.	i.p.	3,65
Wagner O.S.	"	N.S.	i.p.	58
T241 sarcoma	"	i.p.	i.p.	59
KHT fibrosarcoma	"	s.c.	i.p.	66
Mecca lymphosarcoma	"	N.S.	i.p.	58
" "	"	i.p.	i.p.	59

Table 5 (continued)

Tumor	Host	Implant[1] Site	Treatment[1] Route	References
B. 1. (a) continued				
Zimmerman ependymoblastoma	Mouse	i.c.	i.p.	67,68
" (Subline A)	"	i.c.	i.p.	68,69
Glioma 26	"	i.c.	i.p.	68,69,70
Glioma 261	"	i.c.	i.p.	68,69,70
Ehrlich ascites	"	i.p.	i.p.	3,58,59,71
Taper hepatoma (ascites)	"	i.p.	i.p.	58
Krebs II ascites	"	i.p.	i.p.	58
KiMSV	"	i.p.	s.c.	72
"	"	s.c.	i.p.	72
C3H mammary[3]	"	s.c.	i.p.	5,73
LPC-1	"	i.p.	s.c.	74
(b) Rat				
Flexner-Jobling carcinoma	Rat	N.S.	N.S.	58
Iglesias ovarian carcinoma	"	N.S.	i.p.	58
Iglesias adrenal carcinoma	"	N.S.	i.p.	58
Walker 256	"	N.S.	i.p.	58
"	"	i.p.	i.p.	59
"	"	i.c.	i.p.	3
Sugiura-Brown fibrosarcoma	"	N.S.	i.p.	58
Murphy-Sturm lymphosarcoma	"	s.c.	i.p.	3
Jensen sarcoma	"	N.S.	i.p.	58
Yoshida sarcoma	"	N.S.	i.p.	58
9L sarcoma	"	i.c.	i.p.	75
Glioma	"	i.c.	i.p.	76,77

Table 5 (continued)

Tumor	Host	Implant[1] Site	Treatment[1] Route	References
(c) Hamster				
Adenocarcinoma of duodenum, kidney, pancreas, and prostate	Hamster	s.c.	i.p.	3
Carcinoma of adrenal cortex and endometrium	"	s.c.	i.p.	3
Leiomyosarcoma	"	s.c.	i.p.	3
Osteogenic sarcoma	"	s.c.	i.p.	3
Reticulum cell sarcoma	"	s.c.	i.p.	3
Plasmacytoma	"	s.c. or i.c.	i.p.	3

[1]Implant Site and Treatment Route: N.S. = Not Specified
s.c. = subcutaneous
p.o. = via stomach tube
i.p. = intraperitoneally
i.v. = intravenously
i.c. = intracerebrally
i.m. = intramuscularly

[2]DMBA-induced.

[3]First-generation transplant from spontaneous tumor.

Table 6

Published Reports of Therapeutic Activity of CCNU against Experimental Tumors

Tumor	Host	Implant[1] Site	Treatment[1] Route	References
A. Leukemias and Lymphomas				
1. Transplanted				
(a) Mouse				
L1210	Mouse	i.p.	i.p.	5,11,12,13,15,16, 26,37,38,78
"	"	i.p.	p.o.	11
"	"	i.p.	s.c.	79
"	"	i.v.	i.p.	5,26,78,80,81
"	"	s.c.	s.c.	29
"	"	s.c.	p.o.	29
"	"	i.c.	i.p.	5,11,37,38
"	"	i.c.	p.o.	5,11
AKR	"	i.v.	i.p.	82
2. Spontaneous				
(a) Mouse				
AKR	Mouse	Spont.	i.p.	53,55
B. Solid Tumors				
1. Transplanted				
(a) Mouse				
C3H Breast[2]	Mouse	s.c.	i.p.	5,73
Renal cortical adenocarcinoma	"	under renal capsule	i.p.	83
Lewis lung	"	s.c.	i.p.	5,61,62
" "	"	i.v.	i.p.	5,61
B16 melanoma	"	s.c.	i.p.	5, 96
" "	"	i.p.,i.m., or i.c.	i.p.	63
Ependymoblastoma	"	i.c.	i.p.	84

Table 6 (continued)

Tumor	Host	Implant[1] Site	Treatment[1] Route	References
B. 1. (a) continued				
Zimmerman ependymoblastoma A	Mouse	i.c.	i.p.	69,85
"	"	i.c.	i.m.	86
Glioma 26	"	i.c.	i.p.	69,84,85
"	"	i.c.	i.m.	86
Glioma 261	"	i.c.	i.p.	69,84,85
"	"	i.c.	i.m.	86
Glioma[3]	"	i.c.	i.p.	87
(b) Rat				
9L Sarcoma	Rat	i.c.	i.p.	75
(c) Hamster				
Glioma[3]	Hamster	i.c.	i.p.	87
Astrocytoma[4]	"	i.c.	i.p.	87

[1]Implant Site and Treatment Route: i.p. = intraperitoneally
p.o. = via stomach tube
s.c. = subcutaneously
i.v. = intravenously
i.c. = intracerebrally
i.m. = intramuscularly

[2]First-generation transplant from spontaneous tumor.

[3]Rous sarcoma virus-induced.

[4]SV-40 polyoma virus-transformed astrocytes.

Table 7

Published Reports of Therapeutic Activity of MeCCNU against Experimental Tumors

Tumor	Host	Implant[1] Site	Treatment[1] Route	References
A. Leukemias and Lymphomas				
1. Transplantable				
(a) Mouse				
L1210	Mouse	i.p.	i.p.	5,37,38
"	"	i.p.	s.c.	79
"	"	i.c.	i.p.	5,37,38
P388	"	i.p.	i.p.	88
2. Spontaneous				
(a) Mouse				
AKR	Mouse	Spont.	i.p.	53,55
B. Solid Tumors				
1. Transplantable				
(a) Mouse				
C3H Mammary[2]	Mouse	s.c.	i.p.	5
" "	"	i.v.	i.p.	88
Lewis lung	"	s.c.	i.p.	5,61,62,89
" "	"	i.v.	i.p.	5,61
B16 Melanoma	"	s.c.	i.p.	5,90,91,92,96
" "	"	i.c.	i.p.	88
Colon 26	"	s.c.	i.p.	88
Colon 36	"	s.c.	i.v.	88
Colon 38	"	s.c.	i.p.	88
(b) Rat				
9L Sarcoma	Rat	i.c.	i.p.	75

[1]Implant site and Treatment route: i.p. = intraperitoneally
s.c. = subcutaneously
i.c. = intracerebrally
i.v. = intravenously

[2]First-generation transplant from spontaneous tumor.

CCNU and MeCCNU

Compared to the number of reports on BCNU activity, fewer reports of therapeutic activity against experimental tumors treated with CCNU or meCCNU have appeared, probably because these compounds were available more recently than BCNU. As would be expected from the early indicated superiority of CCNU over BCNU against ic implanted tumors, a number of ic implanted primary tumors of the CNS in mice and hamsters, as well as sarcomas, melanomas, and leukemias of rats and mice, have been studied, and CCNU has been shown to have therapeutic activity (table 6). CCNU is also a broad-based and highly active anticancer drug against experimental tumors.

The history of experimental anticancer studies with meCCNU follows a similar pattern. Its early activity against established LL carcinoma indicated studies against other experimental solid tumors with a history of limited response to other anticancer agents. C3H mammary tumor, B16 melanoma, and a number of transplantable colon tumors, as well as LL carcinoma (all possible but not objectively established experimental models for predicting drug response of the major types of high mortality tumors of man with limited responsiveness to available anticancer drugs), have been observed to be sensitive to treatment with meCCNU (table 7). Based on reported activity when used alone, much additional work with combination chemotherapy and combined-modality therapy using meCCNU is indicated.

REPORTED THERAPEUTIC SYNERGISM WITH BCNU, CCNU, OR MECCNU PLUS OTHER AGENTS OR TREATMENT MODALITIES AGAINST EXPERIMENTAL TUMORS

Because maximal destruction or removal of tumor cells with acceptable toxicity to the host is the goal of cancer treatment, and since consistent and predictable "cure" of clinically recognized (advanced) neoplastic disease of man or animals by single-drug treatment has rarely been achieved (80,98), combination chemotherapy with or without other treatment modalities (surgery, radiation therapy, or immunotherapy) is indicated. In selecting drugs for combination chemotherapy trials the following two principles should obtain, if possible: (*a*) the drugs should be qualitatively different in toxicity for vital normal cells and should have different biochemical mechanisms of action against tumor cells; and (*b*) tumor cells resistant to one drug in the combination should not be cross-resistant to the other drug(s).

TOXICITY FOR VITAL NORMAL CELLS OF BCNU, CCNU, OR MECCNU IN COMBINATION WITH OTHER ANTICANCER AGENTS

Less than additive toxicity for vital normal cells in mice has been observed for BCNU, CCNU, and meCCNU in combination with a number of other anticancer agents (81,102) (table 8). The combination toxicity index (CTI) (102) was determined as follows: (*a*) the doses of individual drugs used in a combination toxicity study were tabulated as decimal fractions of the previously determined LD10s for the same treatment schedule; (*b*) the decimal fractions of the LD10s of the individual drugs used in the drug combination were added, and the sums were plotted against observed mortality (at $\geq$ 15 days after last treatment) on log-probit paper; and (*c*) the LD10 (sum) of the drug combination was read from a line of best fit. A significant and reproducible CTI of < 1.0 indicates greater than additive toxicity, a CTI of 1.0 indicates additive toxicity, and a CTI of > 1.0 indicates less than additive toxicity for vital normal cells. A high CTI is not essential for demonstrated therapeutic synergism, but it is an asset to be sought in planning combination chemotherapy trials, since most two-drug combinations reported to be therapeutically synergistic for one or more experimental tumors have CTIs of ≥ 1.3 (81).

Table 8

Combination Toxicity Indices of BCNU, CCNU, or MeCCNU plus Other Anticancer Drugs in BDF1 Mice

Drug Combination	Treatment Schedule	Combination Toxicity Index
BCNU + VCR	Simult., single doses	ca 1.9
BCNU + 6-MeMPR	Offset, qd x 15; single dose	ca 1.8
BCNU + MeCCNU	Simult., single doses	1.6
MeCCNU + Bleo	Overlap, qd x 10 + days 7 & 11	1.6
BCNU + HU	Simult., q3hr x 8; q4d x 4	1.6
MeCCNU + Adria	Offset, qd x 8 + single dose	1.5
MeCCNU + CPA	Simult., single doses	1.5
MeCCNU + 5-FU	Simult., q4d x 3	1.5
CCNU + Bleo	Overlap, qd x 10 + days 3 & 10	ca 1.5
MeCCNU + BIC	Simult., single doses	1.4
BCNU + prednisone	Simult., single doses	ca 1.4
BCNU + CPA	Simult., single doses	1.2-1.4
BCNU + Ara-C	Simult., q3hr x 8; q4d x 4	1.3
MeCCNU + 5-FU	Simult., q4d x 3	1.3-1.5
CCNU + Ara-C	Simult., q3hr x 8; q4d x 4	1.3
BCNU + 5-FU	Simult., single doses	ca 1.2
CCNU + BIC	Simult., single doses	1.1
CCNU + L-PAM	Simult., single doses	1.1
CCNU + CPA	Simult., single doses	ca 1.1
BCNU + DIC	Simult., single doses	0.75
CCNU + DIC	Simult., single doses	0.45

Table 9

Published Reports of Therapeutic Synergism with BCNU plus Other Agents or Treatment Modalities against Experimental Tumors

Tumor	Host	Implant Site	Treatment Route of BCNU	BCNU plus	References
A. Leukemias and Lymphomas					
1. Transplanted					
(a) Mouse					
L1210	Mouse	s.c.	N.S.	MTX	27
"	"	s.c.	s.c.	"	32,34
"	"	i.c.	N.S.	"	27
"	"	s.c.	s.c.	Ara-C	36
"	"	s.c.	s.c.	Ara-C + DIC	93
"	"	s.c.	s.c.	Ara-C + DIC + MTX	93
"	"	i.p.	i.p.	Vit. A alcohol	17,18
"	"	i.p.	N.S.	Vit. A alcohol	94
"	"	i.p.	i.p.,s.c.*	Vit. A alcohol + Caffeine*	18
"	"	N.S.	i.p.,s.c.*	Chlorpromazine + Caffeine*	95
"	"	i.p.	i.p.,s.c.*	"	22
"	"	N.S.	i.p.,s.c.*	Prochlorperazine + Caffeine*	95
"	"	N.S.	i.p.,s.c.*	Chlordiazepoxide + Caffeine*	95
"	"	i.p.	s.c.	Tween 80	25
"	"	i.p.	i.p.	CPA	20
"	"	i.v.	i.p.	"	26
L5178Y	"	i.c.	N.S.	L-Asparaginase	40
P1534	"	i.p.	i.p.	CPA	80
AKR	"	i.v.	i.p. or i.v.	"	82
"	"	i.v.	i.p.	A-4942	82
"	"	i.v.	i.p.	A-4828	82
"	"	i.v.	i.p.	BIC	82
"	"	i.v.	i.p.	5-FU	82
"	"	i.v.	i.p.	Amphotericin B	48,49

Table 9 (continued)

Tumor	Host	Implant Site	Treatment Route of BCNU	BCNU plus	References
2. Spontaneous					
(a) Mouse					
AKR	Mouse	Spont.	i.p.	CPA	53,56
"	"	Spont.	i.p.	PalmO-ara-C	53,55
B. Solid Tumors					
1. Transplanted					
(a) Mouse					
KHT Fibrosarcoma	Mouse	s.c.	i.p.	CPA	66
Colon 26	"	i.v.	i.v.,s.c.*	FUdR*	88
EMT6 Mammary	"	s.c.	i.p.	Irradiation	60
C3H Mammary 44	Mouse	s.c.	i.p.	Surgery	Footnote 10
(b) Rat					
Glioma	Rat	i.c.	i.p.	Surgery	77

Implant site and Treatment route: N.S. = Not Specified
s.c. = subcutaneously
i.c. = intracerebrally
i.p. = intraperitoneally
i.v. = intravenously

*These agents given by the route indicated by an asterisk; other agents given as indicated in "Treatment Route" column.

Table 10

Published Reports of Therapeutic Synergism with CCNU plus Other Agents against Experimental Tumors

Tumor	Host	Implant Site	Treatment Route	CCNU plus	References
A. Leukemias and Lymphomas					
1. Transplanted					
(a) Mouse					
L1210	Mouse	i.v.	i.p.	Ara-C	26,78
"	"	i.v.	i.p.	BIC	80,81
AKR	"	i.v.	i.p.	CPA	82
2. Spontaneous					
(a) Mouse					
AKR	Mouse	Spont.	i.p.	Prednisone, VCR, and PalmO-ara-C	53,55,56

Implant Site and Treatment Route: i.v. = intravenously
i.p. = intraperitoneally

THERAPEUTIC SYNERGISM OF BCNU, CCNU, OR MECCNU WITH OTHER DRUGS OR TREATMENT MODALITIES AGAINST ANIMAL TUMORS

Therapeutic synergism[9] has been reported against a wide variety of experimental tumors treated with BCNU, CCNU, or meCCNU in combination with other anticancer drugs or treatment modalities. These reports are listed in tables 9–11. I will confine my discussion of therapeutic synergism to drug or modality combinations in which improved cure rates have been seen when treatment was delayed until tumor staging was advanced. Usually, the tumors were grossly apparent and not curable or had high curative failure rates if single-drug or -modality treatment was used alone.

Single-Modality (Drug) Treatment

In 1967 we reported that some mice with a total body burden of $> 10^8$ widely disseminated viable L1210 leukemia cells could be cured by treatment with CCNU plus cytosine arabinoside (ara-C) (78) when treatment was delayed until 24 hours before the median day of death in untreated controls, and a positive diagnosis could be made based on leukemic cells in the circulating blood. At about the same time, Valeriote et al (82) reported high cure rates of advanced, transplanted AKR leukemia with combinations of BCNU plus CPA, and we have readily confirmed these results (26). Medoff and associates (48,49) have also reported significant cure rates of advanced, transplanted AKR leukemia with BCNU plus amphotericin B, which we have also confirmed.

The cure of advanced-staged solid tumors with BCNU or meCCNU plus other drugs or treatment modalities has been repeatedly observed and is of the greatest significance for future progress. Lin and Bruce (66) reported significant cure rates of transplanted KHT fibrosarcoma in C3H mice when treatment was delayed until individual tumors were about 500 mg in size and Mayo et al (61) reported cure of LL carcinoma (from 400 to 1000 mg in size) with meCCNU used alone or in combination with CPA.

Combined-Modality Treatment (Surgical Adjuvant Chemotherapy)

Perhaps the greatest progress in potentially practical experimental cancer therapy using nitrosoureas has been made with surgical adjuvant chemotherapy of advanced, metastasized solid tumors, commonly considered to be markedly drug resistant and staged beyond high surgical cure rates at the start of treatment. Mayo et al (61) extended the work of Karrer and Humphreys (4) who reported that LL carcinoma could be cured by surgical removal of the primary tumor followed by treatment with CPA when surgical removal of the primary tumor was delayed until most tumors (about 500-mg primary tumors) had metastasized, and could not be cured by surgery alone. Mayo et al treated progressively more advanced tumors with meCCNU alone or combined with CPA following noncurative surgery. They reported that high cure rates could be obtained with surgery followed by meCCNU plus CPA when surgery was delayed until primary tumors in mice were about 2 g in size and metastatic disease was advanced (Day 16 after implant and > 1 week after metastatic spread to the lungs from the primary tumor). There was 100% curative failure rate with either surgery or chemotherapy used alone (61).

Other solid tumors of mice which are markedly refractory to drug treatment, especially if advanced at the start of therapy, are B16 melanoma, C3H mammary tumors, and transplantable carcinogen-induced colon tumors. Recently published reports and unpublished results from the Southern Research Institute indicate marked progress in combined-modality treatment of these tumors.

B16 Melanoma

Griswold (92) has reported curative surgical adjuvant chemotherapy of B16 melanoma with me-

[9] *Therapeutic synergism:* Two drugs in combination (or drug[s] plus some other therapeutic modality) will result in therapeutic effectiveness greater than that which can be achieved when either drug or modality is used alone under identical conditions of treatment.

CCNU 24 hours after surgery in tumor-bearing animals, staged so that about 80% had metastasized at the time of surgery (80% surgical curative failure rate). Figure 3 is adapted from this recent report. Surgical removal of the primary tumor with this consistently metastasizing tumor resulted in a 20% long-term cure rate. A single dose of meCCNU at about the LD10 level increased the lifespan but produced no cures. However, when the same dose of meCCNU was administered 1 day after surgery, a 70% long-term cure rate was obtained.

C3H Mammary Tumor (Line 44)

Line 44 of the transplanted C3H mammary tumor consistently metastasizes from the sc implanted primary tumor, and low cure rates (0–30%) following surgical removal of primary tumors ranging in size from 0.3 to 1.3 g are usually experienced. In figure 4 marked therapeutic activity of BCNU shortly after noncurative surgical removal of the primary tumor is shown.[10] Surgery alone resulted in no cures but did increase the median lifespan modestly. BCNU alone had no therapeutic effect, but surgery followed by BCNU treatment 1 day later gave a marked ILS of dying animals (about 200% ILS in dying animals) and a 30% cure rate. The influence of disease staging on cure rates with surgical adjuvant chemotherapy of C3H mammary tumors is shown in figure 5.[10]

Surgical removal of the primary tumor on Day 11 after implant gave a 27% cure rate and a marked increase in median lifespan of dying animals. Treatment with BCNU plus CPA without surgery increased lifespan by about 100% but produced no cures. Surgery plus chemotherapy, however, resulted in 100% cures. If surgery was delayed until Day 14 after implant, the surgical cure rate dropped to about 5% and the surgery plus chemotherapy cure rate dropped from 100% to 45%. The median lifespan of dying animals, however, was significantly extended over that obtained with either modality used alone.

Colon Tumor (Line 26)

The effectiveness of meCCNU plus 5-FU as a chemotherapeutic adjuvant to surgery with this metastatic carcinogen-induced colon tumor (103) is illustrated in figure 6 (92). Surgery alone on Day 15 after implant resulted in a very low cure rate, and meCCNU plus 5-FU alone showed essentially no therapeutic activity. However, 5 days after surgery meCCNU plus 5-FU as adjuvant chemotherapy increased the lifespan of dying animals about 200% and resulted in a 40% cure rate.

The data reported with LL carcinoma (61) and those shown in figures 3–6 with B16 melanoma, C3H mammary tumor, and colon 26 (which are representative of many similar data from a number of separate experiments) clearly indicate that BCNU and meCCNU are very active as surgical adjuvants either alone or with other selected anticancer drugs. These observations are particularly pertinent if considered in the light of (*a*) tumors considered to be refractory to drug treatment, (*b*) the importance of tumor staging to cure rates, which clearly reflects the influence of body burden of tumor cells on chemotherapeutic effectiveness, and (*c*) the possible relationship of both the tumor models and the effective drugs to very promising reports on the results of clinical studies with surgical adjuvant chemotherapy of breast carcinoma and osteogenic sarcoma, and combination chemotherapy of advanced tumors of the gastrointestinal tract in man.

DRUG RESISTANCE: ACTIVITY OF BCNU AND CCNU AGAINST TUMOR CELLS SELECTED FOR RESISTANCE TO OTHER ANTICANCER AGENTS

As indicated above, anticancer drugs probably should not be used in experimental or clinical drug combinations if tumor cells selected for resistance to one drug are known to be cross-resistant to the other drug(s) in the combination. Lack of cross-resistance is to be sought in drug selection. Reported sensitivity to BCNU or CCNU of tumor cells selected for resistance to other anticancer agents is shown in table 12. Of great interest and importance is the indication from these reports that at least some tumor cells selected for resistance to some antifols, some purine antagonists, some pyrimidine antagonists, some inhibitors of DNA polymerase, and some other alkylating agents retain sensitivity to BCNU or CCNU. This further indicates consideration of nitrosoureas with demonstrated antitumor activity for inclusion in drug combinations being planned in searches for therapeutically synergistic drugs in both experimental and clinical trials. The additive or less than additive toxicity for vital normal cells with combinations of BCNU, CCNU, or meCCNU plus other chemical classes of anticancer agents (except DTIC) (table 8), and the observed therapeutic synergism with other drugs against a wide variety of experimental tumors (tables 9–11), support this premise.

[10]Corbett TH, and Griswold DP, Jr. Unpublished data; Southern Research Institute.

Table 11

Published Reports of Therapeutic Synergism with MeCCNU plus Other Agents or Treatment Modalities against Experimental Tumors

Tumor	Host	Implant Site	Treatment Route	MeCCNU plus	References
A. Leukemias and Lymphomas					
1. Transplanted					
(a) Mouse					
P388	Mouse	i.p.	i.p.	5-FU	88
2. Spontaneous					
(a) Mouse					
AKR	Mouse	Spont.	i.p.	CPA	53,55,56
"	"	Spont.	i.p.	PalmO-ara-C	53,55
"	"	Spont.	i.p.	PalmO-ara-C + CPA	53,55,56
"	"	Spont.	i.p.	CPA + MTX	56
"	"	Spont.	i.p.	CPA + Adria	56
"	"	Spont.	i.p.	Prednisone, VCR, and PalmO-ara-C	53,55,56
"	"	Spont.	i.p.	VCR + Prednisone + CPA	56
"	"	Spont.	i.p.	CPA + PalmO-ara-C + 6-TG	56
B. Solid Tumors					
1. Transplanted					
(a) Mouse					
Colon 38	Mouse	s.c.	i.p.,i.v.*	5-FU*	88
Colon 26	"	s.c.	i.v.	Surgery	Footnote 10; 97
"	"	s.c.	i.p.,i.v.*	Surgery + 5-FU*	92

Table 11 (continued)

Tumor	Host	Implant Site	Treatment Route	MeCCNU plus	References
B. 1. (a) continued					
C3H Mammary	Mouse	s.c.	i.p.	CPA	80
Lewis lung	"	s.c.	i.p.	CPA	61
" "	"	s.c.	i.p.	Surgery	61
" "	"	s.c.	i.p.	Surgery + CPA	61
B16 Melanoma	"	s.c.	i.p.	CPA	80,91,92,98
" "	"	s.c.	i.p.	Surgery	92
" "	"	s.c.	i.p.,s.c.*	Adria*	91
(b) Rat					
Breast carcinoma[1]	Rat	Primary	i.p.,s.c.*	Dromostanolone propionate*	91
(c) Hamster					
Melanotic Melanoma	Hamster	s.c.	i.p.	L-PAM	91
Osteogenic Sarcoma	"	s.c.	i.p.	CPA	91

Implant Site and Treatment Route: i.p. = intraperitoneally
s.c. = subcutaneously
i.v. = intravenously

[1] DMBA-induced.

*These agents given by the route indicated by an asterisk; other agents given as indicated in "Treatment Route" column.

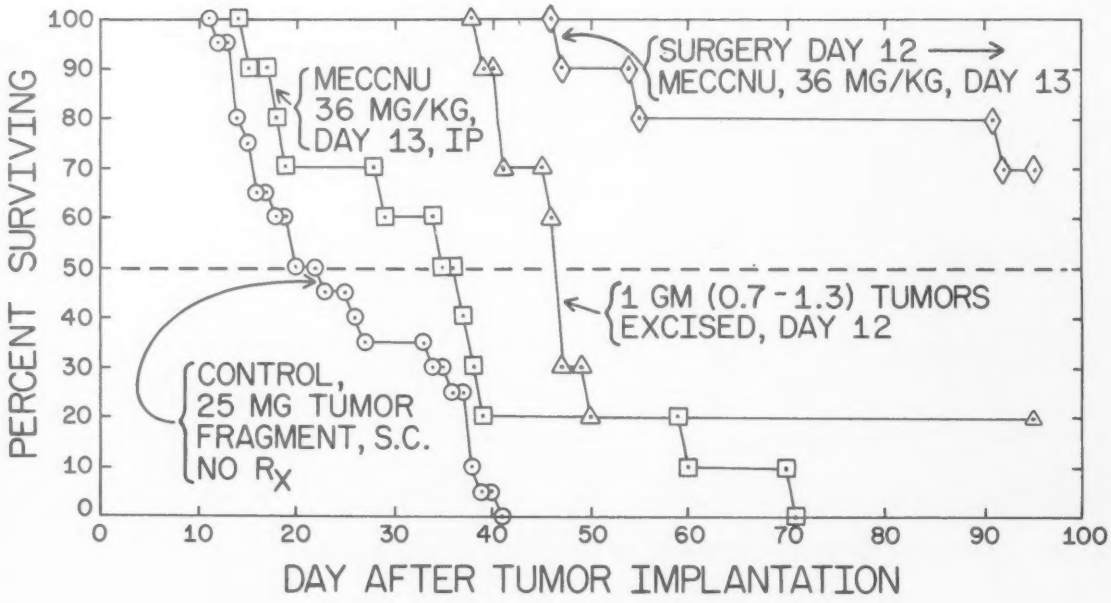


FIGURE 3.—Curative surgical adjuvant chemotherapy of B16 melanoma in BDF_1 mice.

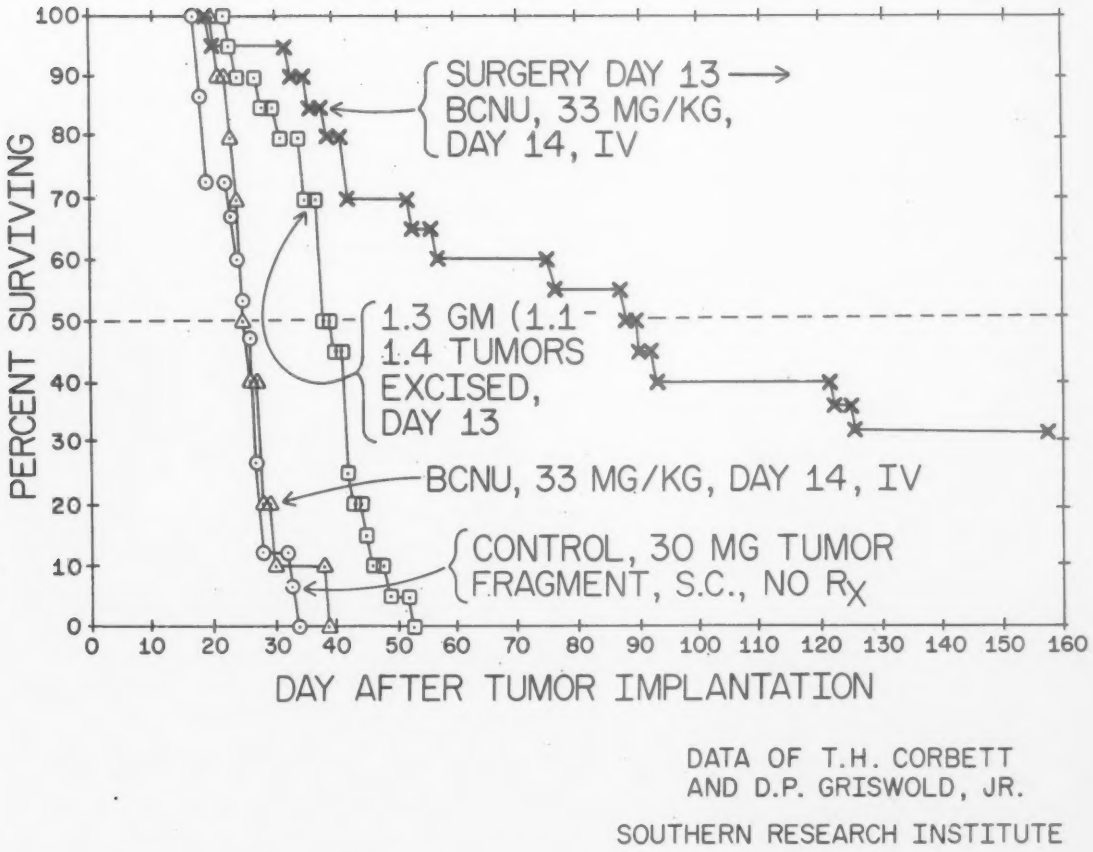
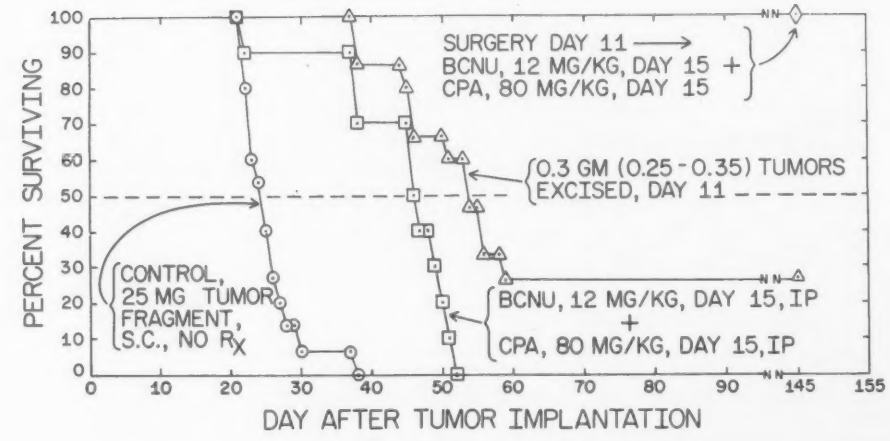


FIGURE 4.—Surgical adjuvant chemotherapy of metastatic C3H mammary tumor, line 44, in C3H mice.



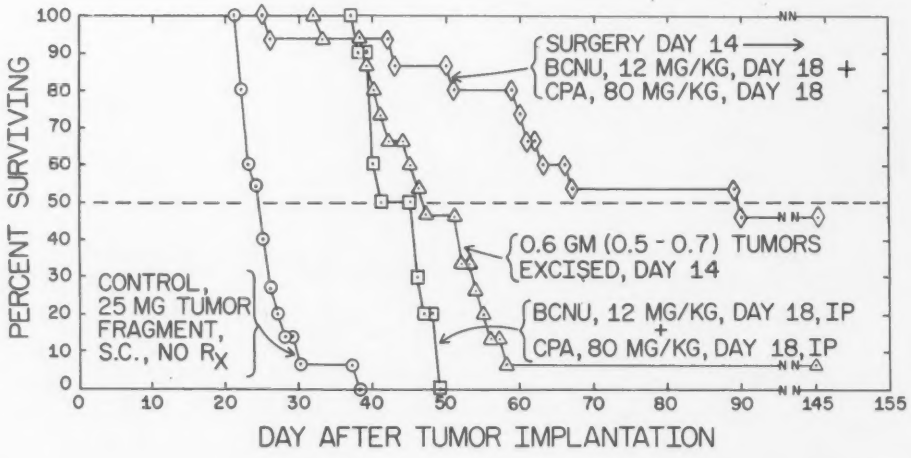


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FIGURE 5.—Surgical adjuvant chemotherapy of metastatic C3H mammary tumors, line 44, in B6C3F, mice.

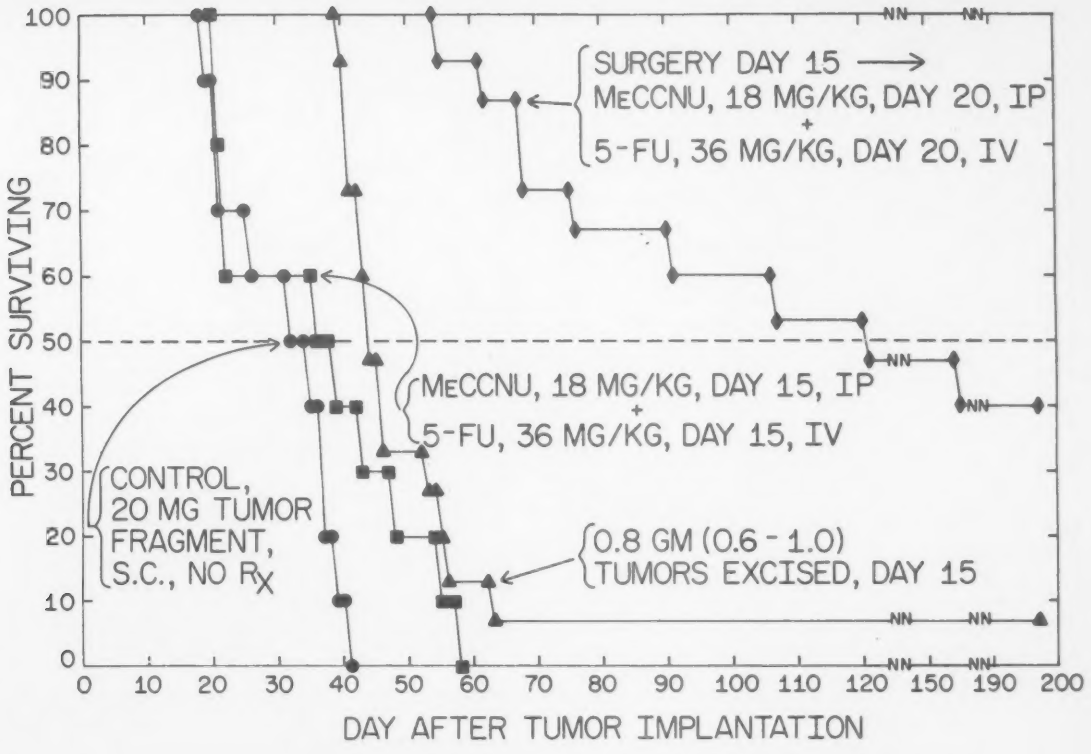

FIGURE 6.—Surgical adjuvant chemotherapy of colon tumor, line 26, in BALB/c mice.

SELECTION OF BCNU- OR MECCNU-RESISTANT SUBLINES OF LL CARCINOMA, B16 MELANOMA, AND L1210 LEUKEMIA: OBSERVATIONS ON THEIR SENSITIVITY TO OTHER ALKYLATING AGENTS

In 1962, Schmidt (104) reported that his observations that a number of transplantable rat tumors selected for resistance to melphalan (L-PAM) or sarcolysin (DL-PAM) were cross-resistant to a number of other different alkylating agents ". . . provide strong support for the concept advanced repeatedly by other investigators that many, if not all, alkylating agents have a basically similar mode of action." Twelve years later, Connors (105) wrote, "From their chemical properties, all the clinically used alkylating agents would be expected to react in a similar way, and very detailed studies of their toxicity and spectrum of antitumor action in animals and man leave no doubt that, while there may be variations in their selectivity of antitumor action, all the alkylating agents have basically similar properties. The development of resistance to one alkylating agent is likely to mean that there will be a cross-resistance to all others; this has been seen in a number of experimental studies on resistance." Some experimental data acquired more recently may be interpreted to indicate that this concept of common biologic activity of all alkylating agents may no longer be tenable. Present knowledge indicates that there are at least four different chemical classes of alkylating agents with different alkylating moieties and biologic mechanisms of action (at least in the mouse) against both vital normal and tumor cells. These are: (*a*) the bis(2-chloroethyl)phosphorodiamidates (eg, CPA); (*b*) the 5-(3,3-disubstituted-1-triazeno)imidazole-4-carboxamides (eg, DTIC and BIC); (*c*) the 1,3-disubstituted-1-nitrosoureas (eg, BCNU, CCNU, and meCCNU); and (*d*) the aromatic nitrogen mustards (eg, L-PAM) (101, 105–108).[11]

[11]Wheeler GP. Personal communication; Southern Research Institute.

Table 12

Drug-Resistant Experimental Tumors Reported to be Sensitive to BCNU or CCNU in Vivo

Tumor	Resistant to	Implant[1] Site	Treatment[1] Route BCNU	Route CCNU	References
A. Leukemias and Lymphomas					
1. Transplantable					
(a) Mouse					
L1210	MTX	i.c.	s.c.		31
"	"	i.v.	i.p.		3
"	"	s.c.	s.c.		31
"	6-MP	i.p.	p.o.		3
"	"	i.c.	p.o.		3
"	MTX, 6-MP & CPA	s.c.	s.c.		31
"	NSC-38280[2]	i.v.	i.p.		3
"	"	i.c.	i.p.		3
"	DIC	i.p.	i.p.		16
"	"	i.p.		i.p.	16
"	Ara-C	i.p.	i.p.		99
"	CPA	i.p.	i.p.		This report
P815	5-FU	i.p.	i.p.		3
B. Solid Tumors					
1. Transplantable					
(a) Hamster					
Pla 1	CPA	s.c.	i.p.		3
(b) Rat					
AH-44[3]	HN2[3]	N.S.	i.p.[4]		100

[1]Implant site and Treatment route: i.c. = intracerebrally
s.c. = subcutaneously
i.v. = intravenously
i.p. = intraperitoneally
p.o. = via stomach tube
N.S. = Not Specified

[2]NSC-38280 is a terephthalanilide.

[3]AH-44 is an ascites hepatoma, naturally resistant to HN2.

[4]HN2 or BCNU alone was ineffective; HN2 plus BCNU gave long-term survivors.

MeCCNU-Resistant LL Tumors

We have repeatedly observed that established LL tumors which regress under optimal treatment with meCCNU and then reappear (fig 2) are markedly resistant to treatment with meCCNU (LL/meCCNU) but retain sensitivity to CPA very similar to that of the parent meCCNU-sensitive tumor (LL/0) from which they were derived.[12] Data from a representative experiment showing these responses are shown in figure 7. It is apparent that the LL/0 tumor is comparably sensitive to both meCCNU and CPA under single-dose LD10 treatment (not the optimal schedule for meCCNU), and the LL/meCCNU line is markedly resistant to meCCNU but retains sensitivity to CPA, apparently about the same as the parent LL/0.

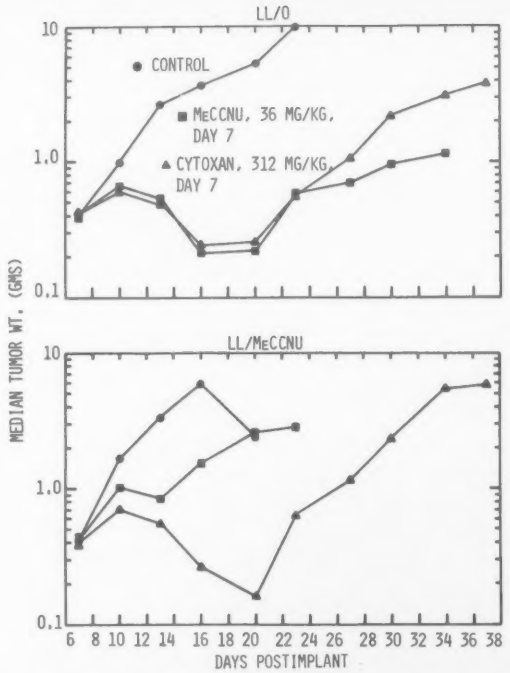

FIGURE 7.—Response of the parent meCCNU-sensitive LL (LL/0) and a subline of LL (LL/meCCNU) obtained after single-course (2 doses, 1 week apart) treatment of advanced (about 500 mg) LL/0 with meCCNU. (The initial meCCNU treatment of LL/0 caused the primary tumor to regress below the limit of palpation [about 60 mg] but was not curative.)

MeCCNU-Resistant B16 Melanoma

Griswold et al (91) have reported the selection of a markedly resistant subline of B16 melanoma (B16/meCCNU) after three to four passages of this tumor under early (Day 2 after implant) treatment with high single doses (LD10) of meCCNU. That the resistant tumor probably represents the overgrowth under treatment of a naturally occurring mutation to meCCNU resistance is evidenced by the fact that its marked resistance to meCCNU treatment has persisted for more than 30 transplant passages with no further exposure to meCCNU.[13,14] When this meCCNU-resistant line of B16 melanoma and the parent meCCNU-sensitive line of B16 melanoma were compared for sensitivity to CPA, they were observed to be essentially equally sensitive (91).

[12]Schabel FM, Jr, Mayo JG, and Laster WR, Jr. Unpublished data; Southern Research Institute.

[13]Griswold DP, Jr, and Dykes DJ. Personal communication; Southern Research Institute.

[14]This represents maintenance of marked and persistent resistance to meCCNU through ≥ 1500 tumor cell divisions.

Table 13

Selection of a Line of L1210 Leukemia Resistant to BCNU

	Passage No.	Average % ILS of Passage Lines Treated with the Single Doses P.O. of BCNU Indicated		
		25 mg/kg	13 mg/kg	8 mg/kg
L1210/0→	0	387	75	34*
	1	-	53*	30
	2	126	53*	
	3	6*	32	
	4	51	19*	
	5	61	12*	
	6	53*		
	7	0*		
	8	29*		
	9	3*		
	10	1*		
	11	12*		
	12	11*		

Resistant

*Treated group from which ascites were harvested, pooled, and passed to new animals; 10^6 leukemia cells were used in each passage.

The reason for the zigzag path of the passage line was that in the first few passages the highest dose of BCNU left too few ascites leukemia cells for reliable counts and passage.

The BCNU-resistant line (L1210/BCNU) was carried through 21 passages, under treatment at 25 mg/kg, single dose p.o. After the twenty-first passage, no further treatment with BCNU was given. L1210/BCNU has now been carried through more than 400 serial passages without further treatment with BCNU, and it has retained apparently unchanged resistance to BCNU during this period of over 10 years and 4000 or more cell generations. These observations indicate to us that L1210/BCNU probably represents a naturally occurring mutation to BCNU resistance.

BCNU-Resistant L1210 Leukemia

In 1964, we isolated a line of L1210 leukemia resistant to BCNU (L1210/BCNU) under continuous treatment with BCNU (table 13). It is apparent that with L1210 leukemia, as with LL carcinoma and B16 melanoma, tumor cell lines resistant to BCNU can be selected rapidly. DeWys (109) isolated a line of L1210 leukemia resistant to CPA (L1210/CPA) which he kindly provided to us. We compared the sensitivity of L1210/0, L1210/CPA, and L1210/BCNU to L-PAM, CPA, and BCNU. Examples of readily reproducible results from such studies are shown in table 14. Data in this table clearly indicate differences in response of these tumor cell lines to these three different alkylating agents: (*a*) L1210/CPA is completely resistant to CPA but is as sensitive to L-PAM or BCNU as L1210/0; and (*b*) L1210/BCNU is markedly resistant to BCNU but is as sensitive to CPA and L-PAM as L1210/0.

These readily reproducible observations indicate that BCNU, CPA, and L-PAM each have a different biologic mechanism of action on the basis of lack of cross-resistance, at least in the BDF, mouse. Additionally, less than additive toxicity for vital normal cells between BCNU and CPA (table 8) and between CPA and L-PAM (108), and therapeutic synergism of CPA plus L-PAM against L1210 leukemia (107,108), P388 leukemia (108), and Ridgway osteogenic sarcoma (108), further support this conclusion.

Table 14

Sensitivity and Cross-Resistance of L1210/0, L1210/CPA, and L1210/BCNU to L-PAM, CPA, and BCNU

Agent	mg/kg, IP Single Dose Day 2 only	Leukemic Cell Implant, IP	L1210/0 %* ILS	L1210/0 60-Day Survivors	L1210/CPA %* ILS	L1210/CPA 60-Day Survivors	L1210/BCNU %* ILS	L1210/BCNU 60-Day Survivors
L-PAM	13	10^5	85	4/10	25	2/9	100	5/10
		10^4	62	8/10	30	8/10	77	5/10
CPA	312	10^6	100	4/10	0	0/7	185	3/10
		10^5	120	5/10	8	0/9	156	6/10
BCNU	27	10^7	157	1/10	175	7/10	33	0/10
		10^6	110	3/10	177	6/7	28	0/10

*ILS in dying animals only.

INDICATIONS FOR ADDITIONAL TRIALS OF ALKYLATING-AGENT DRUG COMBINATIONS

We reported in 1963 that a line of plasmacytoma No. 1 of hamsters selected for resistance to CPA was cross-resistant to BCNU (2).

Schmidt (104) has observed that the several DL- or L-PAM-resistant rat tumors he had isolated were cross-resistant to BCNU.[15] It now appears that, at least with a number of widely studied transplantable tumors of mice, the concept of a uniform mechanism of action of alkylating agents, against either vital normal or tumor cells, may no longer be tenable. Our most serious problem, of course, is that we don't know whether the tumors of mice, rats, or hamsters reliably predict chemotherapeutic response of any human tumor (98). If the observations on tumors in mice reported here should obtain with some tumors in man, the implications for improved alkylating-agent therapy in man are enormous, since (*a*) less than additive toxicity for normal cells with two-drug alkylating-agent combinations in mice has been seen repeatedly, (*b*) therapeutic synergism of two-drug alkylating-agent combinations is common and quantitatively impressive, and (*c*) tumor cells selected for resistance to members of one class of alkylating agents are clearly as sensitive as the parent cells to members of one or more other classes of alkylating agents. These three concepts are the triumvirate of assets most desirable in drug combinations, and all have been experimentally demonstrated to obtain with combinations of BCNU, CCNU, or meCCNU with other alkylating agents in one or more animal tumor systems.

LIST OF COMPOUNDS

A-4828: NSC-109723; CAS reg. No. 22089–22–1; phosphoric acid ester-diamide, *N,N,N'*-tris-(2-chloroethyl)-*N'-O*-propyl-; trophosphamide

A-4942: NSC-109724; CAS reg. No. 3778–73–2; phosphoric acid ester-diamide, *N*-(2-chloroethyl)-*N'*-(2-chloroethyl)-*N'-O*-propyl-; isophosphamide

Adriamycin: NSC-123127; CAS reg. No. 23214–92–8; adria

Amphotericin B: NSC-527017; CAS reg. No.30652–87–0;(1*R*,3*S*,5*R*, 6*R*, 9*R*,11*R*,15*S*,16*R*,17*R*,18*S*,33*R*,35*S*,36*S*,37*S*)-33-[(3-amino-3,6-dideoxy-β-L-mannopyranosyl)oxy]-1,3,5,6,9,11,17,37-octahydroxy-15,16,18-trimethyl-13-oxo-14,39-dioxabicyclo[33.3.1]nonatriaconta-19,21,23,25,27,29,31-heptane-36-carboxylic acid

L-Asparaginase: NSC-109229; CAS reg. No. 9015–68–3

Azaserine: NSC-742; CAS reg. No. 115–02–6; serine, diazoacetate (ester), L-

BCNU: NSC-409962; CAS reg. No. 154–93–8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-

BIC: NSC-82196; CAS reg. No. 5034–77–5; imidazole-4-carboxamide, 5-[3,3-bis(2-chloroethyl)-1-triazeno]-

Bleomycin: NSC-125066; bleo

Caffeine: NSC-5036; purine, 1,3,7-trimethyl-2,6-dioxo

CCNU: NSC-79037; CAS reg. No. 13010–47–4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-

Chlordiazepoxide: 3*H*-1,4-benzodiazepine, 7-chloro-2-methylamino-5-phenyl-, 4-oxide

Chlorpromazine: phenothiazine, 2-chloro-10-(3-dimethylaminopropyl)-

Cyclophosphamide: NSC-26271; CAS reg. No. 6055–19–2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate; CPA

Cytosine arabinoside: NSC-63878; CAS reg. No. 69–74–9; cytosine, 1-β-D-arabinofuranosyl-, monohydrochloride; ara-C

Dromostanolone propionate: NSC-12198; CAS reg. No. 521–12–0; 5α-androstan-3-one, 17β-hydroxy-2α-methyl-, propionate

DTIC: NSC-45388; CAS reg. No. 4342–03–4; imidazole-4-carboxamide, 5-(3,3-dimethyl-1-triazeno)-; DIC

5-Fluorouracil: NSC-19893; CAS reg. No. 51–21–8; 5-FU

5-FUDR: NSC-27640; uridine, 5-fluoro-2'-deoxy-

Hydroxyurea: NSC-32065; CAS reg. No. 127–07–1; HU

Mechlorethamine: NSC-762; CAS reg. No. 55–86–7; diethylamine, 2,2'-dichloro-*N*-methyl-, hydrochloride; HN2

Melphalan: NSC-8806; CAS reg. No. 3223–07–2; alanine, 3-[*p*-[bis(2-chloroethyl)amino]phenyl]-, monohydrochloride, L-; L-PAM

Methyl-CCNU: NSC-95441; CAS reg. No. 33073–59–5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-; meCCNU

6-Methylmercaptopurine riboside: NSC-40774; CAS reg. No. 7143–67–1; 9*H*-purine, 6-(methylthio)9-β-D-ribofuranosyl-, dihydrate; 6-MeMPR

1-Methyl-3-nitro-1-nitrosoguanidine: NSC-9369; CAS reg. No. 70–25–7; MNNG

1-Methyl-1-nitrosourea: NSC-23909; CAS reg. No. 684–93–5; MNU

6-Mercaptopurine: NSC-755; CAS reg. No. 6112–76–1; purine-6-thiol, monohydrate; 6-MP

Methotrexate: NSC-740; CAS reg. No. 59–05–2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-; MTX

Mitomycin C: NSC-26980; CAS reg. No. 50–07–7

NSC-38280: terephthalanilide, 2-chloro-4',4''-di-2-imidazolin-2-yl-, dihydrochloride

PalmO-ara-C: NSC-135962; CAS reg. No. 31088–06–9; cytosine, 1-β-D-arabinofuranosyl-, 5'-palmitate

Polysorbate 80: NSC-406473; CAS reg. No. PM9005–70–3; sorbitan, monooleate, polyoxyethylene deriv; Tween 80

Prednisone: NSC-10023; CAS reg. No. 53–03–2; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-

Prochlorperazine: NSC-17478; phenothiazine, 2-chloro-10-[3-(4-methyl-1-piperazinyl)propyl]-; dihydrobromide

6-Thioguanine: NSC-752; CAS reg. No. 1832–72–0; purine-6-thiol, 2-amino-; 6-TG

Vincristine: NSC-67574; CAS reg. No. 2068–78–2; leurocristine, sulfate (1:1); VCR

Vitamin A alcohol: NSC-122759; 3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)nona-2,4,6,8-tetraen-1-ol

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Review of Mechanism of Action Studies of the Nitrosoureas

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The biochemical actions of the nitrosoureas have been studied by a large number of investigators and encompass a broad scope of specialized fields. I will be discussing some representative data from some of the published work; my references are not meant to represent a comprehensive presentation of all research.

The basic problem biochemists and molecular pharmacologists have in studying a biologically active drug is relating the chemical activities they find to the biologic activity. With this in mind I will first review the effects of the nitrosoureas (primarily BCNU, CCNU, methyl-CCNU, and chlorozotocin)[2] on both cell survival and cell-cycle progression so that their chemical activities can be considered in the context of these effects.

Effects on Cell Survival

Bhuyan et al have found that in actively proliferating Chinese hamster fibroblast cells, maximal cell kill occurs in cells in late G_1 or early S phase (1). About 3 hours after mitosis, synchronized Chinese hamster fibroblast cells which are somewhere in late G_1 or early S phase are ten to 100 times more sensitive than cells in other phases of the cell cycle. The same pattern of sensitivity was seen by Barranco and Humphrey in mitotically selected Chinese hamster ovary (CHO) cells (2). However, when they used S-phase arrest as the synchronizing method, mid S phase was found to be most sensitive. Drewinko also found mid S phase as the most sensitive time for a human lymphoma cell line.[3]

In vivo, normal hematopoietic cells are less sensitive to BCNU, CCNU, and methyl-CCNU than are either rapidly proliferating marrow cells or tumor cells. Also, van Putten et al found that normal marrow cells survived better than rapidly proliferating marrow cells for all three drugs (3). In a recent paper, Blackett et al described a similar relationship between normal marrow cells and both B16 melanoma and Lewis lung carcinoma (4). Hagemann et al studied the sensitivity of solid forms of the P815X2 mastocytoma.[4] They found that the primary tumor was less sensitive than small splenic and hepatic metastases. Furthermore, the older the primary solid tumor, the less sensitive the cells.

All of this in vivo evidence would support the hypothesis that the nitrosoureas are more effective against proliferating cells than nonproliferating cells. However, there are other reports, including data from Hagemann, that indicate just the opposite, that is, that nonproliferating cells are more sensitive. Hagemann et al found that P815X2 cells in either ascites form or in suspension culture became more sensitive as the age and density of the cell population increased (5). Corroborating this effect Barranco et al found that nondividing CHO cells in culture were more sensitive to CCNU and methyl-CCNU than dividing cells (6). Tobey and Crissman have reported a similar difference (7). These observations have been tempered by the work of Hann et al who have shown that the activity of BCNU was influenced by the concentration and age of the serum in the medium (8). They found that the sensitivity of proliferating cells could be increased by treating them with BCNU in the presence of medium from nonproliferating cultures. In spite of this, all the in vitro cell culture data point to a greater sensitivity for the nonproliferating cells.

Since the cell culture data show the nonproliferating cells to be most sensitive, how then are the in vivo data (which suggest the opposite) explained? It has been speculated that the in vivo results are due to factors other than kinetic ones. For example, the relative lack of resistance of the large primary

[1]Drug Research and Development Program (DR&DP), Division of Cancer Treatment (DCT), National Cancer Institute (NCI), Bethesda, Md.

[2]BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-.

CCNU: NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-.

Methyl-CCNU: NSC-95441; CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-.

Chlorozotocin: NSC-178248; D-glucopyranose, 2-[[[(2-chloroethyl)nitrosoamino]carbonyl]amino]-2-deoxy-.

[3]Drewinko B. Progress Report to the NCI, contract N01-CM-61156, June 1, 1974 to Dec 31, 1975.

*_Reprint requests to:_ Dr. Vincent H. Bono, Jr, Molecular Biology and Methods Section, Laboratory of Medicinal Chemistry and Biology, DR&DP, DCT, NCI, Bg 37, Rm 6B22, National Institutes of Health, Bethesda, Md 20014.

[4]Hagemann RF, Schenken LL, Poulakous L, et al. Metastatic tumor response to chemotherapy: dependence upon site (mode) of growth. Progress Report to the NCI, contract N01-CM-43730, Sept 1973.

P815X2 tumor in vivo may be due to decreased drug accessibility and the relative resistance of marrow cells may relate to some special characteristic of hematopoietic tissue. The cell culture data, therefore, indicate that the killing action of these drugs does not require a cell with active DNA synthesis.

Effects on Cell-Cycle Progression

Cell-cycle progression is arrested in G_2 phase by BCNU, CCNU, and methyl-CCNU (7). In this report from Tobey and Crissman the progression of synchronized CHO cells was measured by flow microfluorometry of cell DNA content. Progression from G_1 to S phase proceeded normally, progression from S phase to G_2 phase was slowed but not stopped, and progression through G_2 phase was arrested. The position of arrest within G_2 phase is of some interest (9). In exponentially growing CHO cells, the G_2 phase is 186 minutes long. The time it takes for the population to stop dividing after adding drug was used as a measure of how early in the G_2 phase the block has occurred. BCNU, CCNU, and methyl-CCNU all blocked in the last one third of the G_2 phase while streptozotocin (NSC-85998) and chlorozotocin appeared to block in early G_2 phase. In summary then, the nitrosoureas as a group kill nondividing cells more effectively than dividing cells, dividing cells are most sensitive to their cytocidal action in early S phase, but the cycle progression of dividing cells is arrested in G_2 phase. This suggests that whatever effects nitrosoureas have on DNA synthesis, they are subtle ones which allow completion of DNA synthesis but perhaps interfere with its function as cells progress through G_2 phase.

Biochemical Effects

With this as a background, I will turn to the reported biochemical actions of the nitrosoureas. As Dr. Montgomery shows in another paper in this issue (10), the chloroethyl nitrosoureas decompose spontaneously with reaction half-lives of approximately 30 minutes. They yield vinyl carbonium ions and chloroethyl carbonium ions which are alkylating agents. They also yield an organic isocyanate which can react with electron-rich groups like primary amino groups to give stable urea derivatives. This latter carbamylation reaction is likely to occur with amino groups of proteins.

The effect of CCNU on DNA synthesis in L1210 leukemia cells in culture was studied by Woolley et al (11). They found a progressive decrease in the rate of DNA synthesis at 10^{-5} M. By comparison, effects on protein synthesis were smaller and transient. Wheeler and Alexander found that the nitrosoureas caused prolonged inhibition of DNA synthesis in a hamster plasmacytoma and in murine Lewis lung carcinoma (12). DNA synthesis in normal tissues, on the other hand, was only transiently inhibited. DeVita et al found that the rate of DNA synthesis in L1210 leukemia cells in vivo was reduced for several generations after exposure to BCNU (13). This effect was much less apparent after treatment with CCNU and methyl-CCNU.

What kinds of detailed biochemical effects on DNA synthesis have been found? First, the alkylating moiety has been shown to react with nucleic acids to modify their structure. Cheng et al studied the binding of CCNU to macromolecules of L1210 cells (14). Binding was due to both carbamylation and to alkylation. They found that RNA, DNA, and protein were all alkylated to about the same extent. Ludlum et al have found that BCNU alkylates polycytidylic acid and poly-guanylic acid but not polyadenylic acid or poly-uridylic acid (15). By identifying the alkylated products they conclude that a chloroethyl carbonium ion is the alkylating species. The production of altered bases by alkylation can be responsible for the lethal action of the nitrosoureas.

There is, however, no evidence that alkylation inhibits DNA synthesis per se. Wheeler and Bowdon found that treating either denatured or native DNA with BCNU did not decrease its template activity in a cell-free DNA-synthesizing system (16). However, they did find that BCNU treatment of the DNA polymerase enzyme inhibited its activity dramatically. In parallel studies with chloroethyl isocyanate, the carbamylating species from BCNU, they concluded that carbamylation of the enzyme, rather than alkylation, is responsible for its activity.

A second kind of effect on DNA has been observed: fragmentation of the DNA molecule. Hilton[5] reports that CCNU produces strand breaks in DNA at a rate similar to that produced by X-radiation. However, unlike X-ray damage which is rapidly repaired, CCNU damage persists. This finding may be explained by the work of Kann et al (17). Using a highly sensitive method for DNA strand breaks, they found that chloroethylisocyanate interferes with repair of DNA damaged by X-rays or by ultraviolet irradiation. They concluded that carbamylation by chloroethylisocyanate, a breakdown product of BCNU, may be responsible for the inhibition of repair. This may be a result of inactivation of repair enzymes.

The carbamylating activity of the nitrosoureas may be responsible for many of their actions. The carbamylation of proteins was shown by Cheng et al

[5]Hilton J. Personal communication. Baltimore Cancer Research Center, DCT, NCI.

(14) and by Wheeler et al (18) to be significant. Woolley et al showed that carbamylation of proteins is selective (11). Chromatography of L1210 leukemia cell histone proteins after exposure to CCNU showed extensive carbamylation of the H1 histone. Carbamylation of other histone proteins and of the nuclear acidic proteins was insignificant by comparison. Kann et al have shown that BCNU interferes with the maturation of ribosomal precursor RNA and with nucleoplasmic messenger-precursor RNA (19). This effect resulted in the persistence of large RNA molecules after BCNU treatment. In this study carbamylation (possibly carbamylation of ribonucleases) was shown to be probably responsible for these effects. Groth et al found that BCNU interfered with the incorporation of carbon from histidine into purines during de novo purine biosyntheis (20). At the same time it increased the incorporation of carbon from both serine and formate. These results suggested an inhibition of one of the steps involved in the one-carbon metabolism of histidine. Although no further information is available, it is possible to speculate that selective carbamylation of these enzymes may be responsible.

Another unusual enzymic effect of BCNU is its stimulating effect on nicotinamide adenine dinucleotide glycohydrolase activity in mouse tumor tissue but not in normal tissue (21). In five tumor types, the enzymatic activity rose four to ten times while there was no increase in a range of normal tissue activities. Neither the role of this effect on nicotinamide adenine dinucleotide metabolism nor the role of carbamylation has been evaluated.

On the basis of a structure-activity study of the nitrosoureas, Wheeler et al have proposed that alkylation is responsible for the therapeutic action and that carbamylation is responsible for the toxic action of these drugs (22). In support of this, Anderson et al have shown that chlorozotocin, which has very low carbamylating activity, produces much less bone marrow toxicity than BCNU in the mouse (23). Chlorozotocin produced a smaller depression in peripheral white blood cell count than BCNU after comparable minimal lethal doses.

The low carbamylating activity of chlorozotocin may be due to the particular organic isocyanate it generates. Since it generates an isocyanate sugar, internal carbamylation with one of the sugar hydroxyl groups may occur. Recently, May et al have reported the metabolic hydroxylation of the cyclohexyl group of CCNU by microsomal enzymes (24). These products will produce hydroxylated organic isocyanates, some of which may have diminished carbamylating activities for the same reason that chlorozotocin does. At this point it can only be speculated that various patterns of metabolism may significantly alter the toxicity of CCNU.

Conclusions

How do all of these varied and potential activities of the nitrosoureas account for their therapeutic effect and their effects on cell survival and cell-cycle progression? Briefly, they can alkylate DNA, prevent the repair of DNA, and alter the structure of RNA and the structure and function of a host of proteins and enzymes. Their effects on DNA structure do not require active DNA synthesis. Perhaps the DNA of nonproliferating cells is even more susceptible to damage than is the DNA of proliferating cells. Their effects on DNA-polymerizing and RNA-metabolizing enzymes may be responsible for the slowing of DNA synthesis and of cell-cycle progression. Perhaps damaged DNA structure prevents cells in the G_2 phase from progressing into mitosis. These are simplistic speculations, but at present they represent our current state of understanding.

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Pharmacology of the Nitrosoureas: An Overview

Vincent T. Oliverio[1,*]

Since the initial clinical and pharmacologic evaluation of BCNU by DeVita and associates (1,2) about 10 years ago, there has been mounting evidence concerning the pharmacology, and particularly the metabolism, of the halogenated alkylnitrosoureas. As a group, BCNU, CCNU, and methyl-CCNU (me-CCNU)[2] have several properties in common: (*a*) they are highly effective against ip and ic L1210 leukemia; (*b*) they are highly lipid soluble which facilitates their rapid transport into cells and across the blood-brain barrier; (*c*) they decompose nonenzymatically at relatively rapid rates especially when exposed to varying conditions of pH, temperature, and media into which they are placed; and (*d*) their biotransformation products are bound to macromolecules through alkylation of nucleic acids and proteins as well as through carbamoylation of proteins in intact cells.

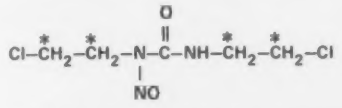
The general pharmacokinetic picture is similar to that of many typical alkylating agents, ie, following drug administration events move very rapidly with extensive drug tissue distribution and metabolism to highly reactive intermediates. How the nature and selectivity of these intermediates relate to antitumor activity and toxicity is still poorly understood, but recent advances in biochemical analytic methodology have shed more light upon these questions.

This brief paper will be confined to the pharmacology of the three nitrosoureas whose structures are shown in figure 1. The asterisks shown in the various positions of each molecule indicate the carbon atoms which have been radioactively labeled to facilitate pharmacologic and mode of action studies.

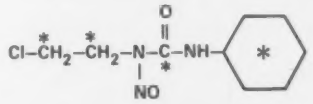
The most consistent organ toxicities of these drugs involve the bone marrow, lymphoid tissue, kidneys, lungs, liver, and gastrointestinal tract. The effects on hepatic, cardiopulmonary, and renal systems are of particular concern. The renal toxicity in monkeys is often unpredictable and nonreversible at high doses. Fortunately, most of these hazards observed in experimental animals have either been manageable or have not been a problem clinically. The clinical toxicity of these agents is covered in some detail by Wasserman (3).

Because of the reactivity of the nitrosoureas in physiologic systems, it is not surprising to find that the chemical and biologic half-lives of these agents are short (table 1). Based on the killing of L1210 leukemia cells injected in mice at various times after drug administration, the biologic half-life is between 15 and 30 minutes for BCNU and 94 minutes for CCNU (4,5). The chemical half-life in vivo of BCNU in the plasma of dogs, monkeys, and man is < 5 minutes (2), while for CCNU (6) it is in the order of 15 minutes. As early as 5 minutes following oral dosage of BCNU, CCNU, or meCCNU in man, no parent drug is detectable in the plasma, although there is a persistence of other drug products (2,7). The biologic half-life data in the mouse appear to be consistent with the conversion of BCNU to an inactive product and to CCNU, a product which retains antitumor activity, since the chemical half-lives of both compounds are similar.

BCNU: 1,3-Bis(2-chloroethyl)-1-nitrosourea



CCNU: 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea



MeCCNU: 1-(2-Chloroethyl)-3-(4-methyl cyclohexyl)-1-nitrosourea

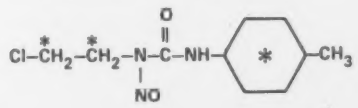


FIGURE 1.—Structures of the nitrosoureas; $\overset{*}{C}$ indicates ^{14}C position.

[1]Experimental Therapeutics Program (ETP), Division of Cancer Treatment (DCT), National Cancer Institute (NCI), Bethesda, Md.

[2]BCNU: NSC-409962; CAS reg. No. 154–93–8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-.

CCNU: NSC-79037; CAS reg. No. 13010–47–4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-.

Methyl-CCNU: NSC-95441; CAS reg. No. 33073–59–5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-.

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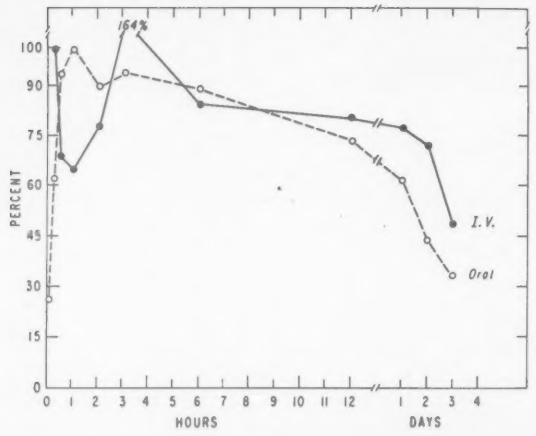


FIGURE 2.—Comparison of average plasma levels of radioactivity in man after oral or iv injection of ^{14}C-BCNU. Values expressed as percent of initial peak level (*see* ref 2).

TABLE 1.—Chemical and biologic half-lives of the nitrosoureas

Half-life	Half-life in mins		
	BCNU	CCNU	MeCCNU
Biologic (L1210 cell kill in vivo)	15–30	94	—
Chemical (plasma in vivo)			
Mouse	< 5	6	5
Dog	< 5	< 15	—
Monkey	< 5	< 15	—
Man (oral)	ND*	ND	ND

*ND = parent drug not detected in plasma.

Figure 2 compares the average plasma levels of radioactivity in man after oral or iv injection of BCNU labeled in the chloroethyl carbons. The values are expressed as percent of initial peak level. The plasma levels of the isotope are prolonged with a half-life of 34 hours for the orally administered drug and a half-life of 67 hours for the iv injected drug. This is most likely attributable to a combination of plasma-protein binding of the drug and enterohepatic recirculation. The initial dip and rise of the plasma levels may also be a result of enterohepatic recirculation and possible release of a lipid-insoluble fraction of the drug into the plasma following initial distribution in tissues. Similar plasma patterns are seen with CCNU and meCCNU.

Figure 3 shows the concurrent plasma-cerebrospinal fluid (CSF) levels of intact CCNU and CCNU plus degradation products in the dog after a single iv dose of the ethylene-labeled drug. In the dog the half-life of the parent drug in CSF and plasma is about 5 minutes for the initial disappearance phase

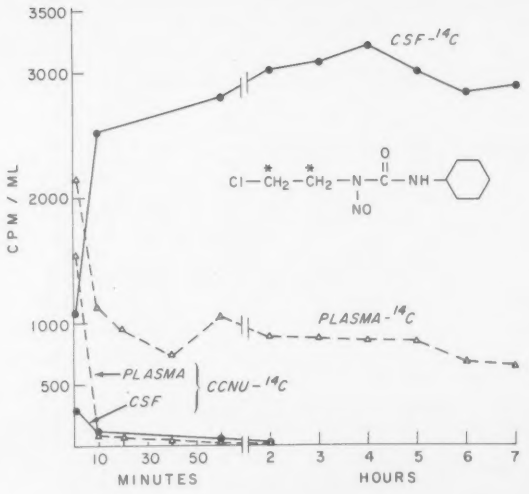


FIGURE 3.—Plasma and CSF levels of CCNU and biotransformation products in a female mongrel dog after a single iv injection of 15 mg/kg ^{14}C-ethylene-labeled parent drug (*see* ref 6).

followed by a second disappearance phase extending over 1 hour. Of particular interest is the rapid entry of CCNU plus degradation products into the CSF. A plasma:CSF ratio of 1:3 was achieved with the ethylene-labeled drug by ½ hour and maintained for a prolonged period of time.

With the cyclohexyl-labeled drug there is a similar rapid entry of CCNU plus degradation products into the CSF, but the plasma:CSF ratio in the dog in this case was only 2:1 (fig 4). The difference between the levels of cyclohexyl- and ethylene-label in the CSF compared to plasma can be accounted for by the plasma-protein binding of the cyclohexyl moiety (which varies between 40% and 60%) and the absence of plasma-protein binding with the ethylene moiety. Thus, the ethylene portion of the molecule has less difficulty in crossing the blood-brain barrier in contrast to the cyclohexyl portion of the molecule.

Figure 5 shows the average plasma levels of radioactivity representing the cyclohexyl, chloroethyl, and carbonyl moieties of CCNU following single oral doses ranging from 30 to 100 mg/m^2 in ten patients who received the drug as part of their early trial. With label in any of the moieties there was prompt absorption and a rapid rise in plasma levels of radioactivity with the average peak plasma levels occurring between 1 and 4 hours. There is also a prolonged disappearance phase exceeding 1 day. No intact drug was detectable in any plasma sample after oral administration. There was also prompt

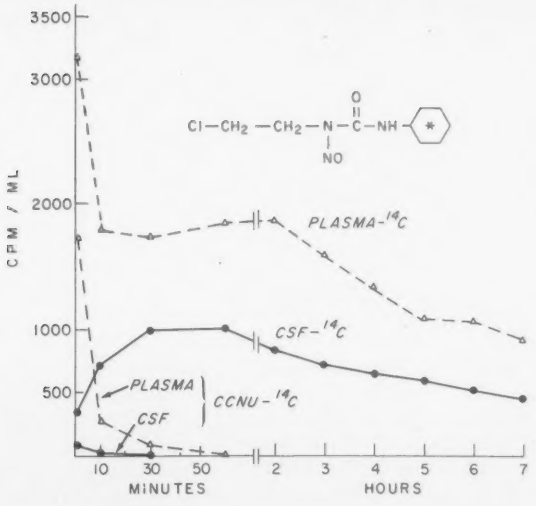


FIGURE 4.—Plasma and CSF levels of CCNU and biotransformation products in a female mongrel dog after a single iv injection of 15 mg/kg ^{14}C-cyclohexyl-labeled parent drug (*see* ref 6).

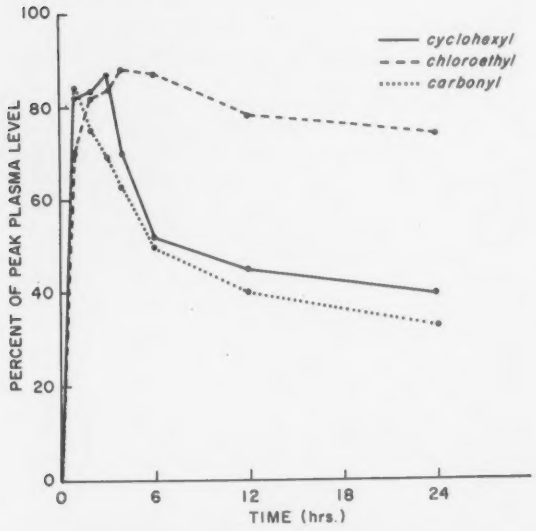


FIGURE 5.—Average plasma levels of radioactivity in 10 patients after orally administered CCNU (30–100 mg/m^2) (*see* ref 7).

entry of degradation products into the CSF of man which closely paralleled the concurrent plasma levels, with the absolute levels being 15%–30% of the concurrent plasma levels. Similar results have been obtained with meCCNU. The activity of these agents in treating central nervous system tumors in animals and man is probably the result of this transport across the blood-brain barrier. The results of plasma-CSF disappearance studies, particularly in man, where CCNU and meCCNU have shown therapeutic benefit, strongly suggest that the intact parent molecule is essentially not responsible for the antitumor and myelosuppressive effects.

The primary route of elimination of the three nitrosoureas and their biotransformation products is through the kidneys, with biliary excretion and reabsorption from the gut also playing a prominent role. Table 2 compares the initial 24-hour urinary recovery of radioactivity derived from the ethylene-labeled nitrosoureas. Fecal excretion amounts to < 5% of the dose and < 10% of the dose appears in expired air.

Table 3 demonstrates that in contrast to a rather homogeneous distribution of drug-derived radioactivity observed in the organs of rodents following a parenteral dose of CCNU, the monkey at 5 days showed appreciable concentration of radioactivity in the liver, bile (~ 1/3 of the specific activity at the time of injection), and kidneys. Despite the high activity in the bile, the intestinal radioactivity was considerably less. In the case of rodents, dogs, and monkeys, the levels of both urinary and biliary excretion of BCNU, CCNU, and meCCNU indicate that both the liver and kidneys are exposed to relatively high concentrations of metabolic degradation products which might be bound and adversely affect cellular function at a later period.

While the pharmacologic distribution and urinary excretion of the nitrosoureas and their products have been extensively studied and some evidence has been presented for their biotransformation in vivo, it has only been within the last few years that a clearer picture has begun to emerge concerning the metabolism of these agents, particularly by microsomal enzymes.

Reed, May, and co-workers at the Oregon State University have studied the metabolism of CCNU in an effort to understand its mode of action and its toxic actions. In 1974 they reported that CCNU is rapidly hydroxylated by rat liver microsomes in three separate ring positions of the cyclohexyl moiety: the 2-position in trace amounts, but more predominantly the 3- and 4-positions (8). The hydroxylation is cytochrome P-450 dependent. In vivo evidence for this hydroxylation is shown in table 4 by data on the urinary excretion of the free and conjugated hydroxy-cyclohexylamine bases following iv injection of ^{14}C-cyclohexyl-CCNU in rats.[3] After 24 hours, 8.0% of the dose was excreted as the free amine and an equal amount in the conjugated form. The breakdown of the different isomers shows that the *cis*-3-hydroxy compound is dominant. In phenobarbital-induced animals there appears to be

TABLE 2.—24-hr urinary excretion of ^{14}C (*see* refs 2, 6, 7)

	Route	% of dose excreted		
		BCNU*	CCNU*	MeCCNU*
Mouse	oral	78	80	71
	ip	78	86	73
Dog	iv	29 (6 hrs)	37	29
Monkey	iv	61	88	ND
Man	oral	49	50	47
	iv	38	ND†	ND

*^{14}C-ethylene.
†ND = not done.

TABLE 3.—Distribution of residual radioactivity in a female rhesus monkey 5 days after a single iv injection of ^{14}C-ethylene-CCNU (10 mg/kg, 18.3×10^6 cpm) (*see* ref 6)

Organ	Specific activity (cpm/g)
Liver	9410
Bile	1180
Kidney	570
Spleen	390
Lung	310
Heart	300
Muscle	240
Small intestine	190
Large intestine	180
Ovary	0
Brain	0

a change in the quantitative distribution of the hydroxylated metabolites with the *cis*-4-hydroxy compound being dominant. A possible explanation is that the induced system contains at least two enzymes capable of cyclohexyl ring hydroxylation. The normal enzyme produces primarily the 3-hydroxy isomer and the induced enzyme produces primarily the *cis*-4-hydroxy isomer. These data appear to be consistent with preliminary urinary excretion data in the monkey where the free and conjugated hydroxylated bases have been identified.[3] The inability to identify cyclohexylamine as a urinary metabolite in the rat suggests that very little CCNU undergoes degradation in vivo prior to ring hydroxylation. The data here appear to indicate that carbamoylation in vivo may not be due to cyclohexylisocyanate per se but to hydroxy-cyclohexylisocyanates which may have different reactivities toward cellular constituents.

BCNU is also a substrate for microsomal enzymes. Hill and co-workers (9) reported that microsomal enzyme of mouse liver, and to a much lesser extent of mouse lung, catalyzes the denitrosation of not only BCNU but also of 1-methyl-1-nitrosourea (MNU)[4] which has been of clinical interest in the Soviet Union. The end product of the reaction in the case of BCNU is 1,3-bis(2-chloroethyl)urea, although the mechanism is not clear. Interestingly, the microsomal denitrosation of BCNU and MNU results in products with negligible antitumor activity whereas hydroxylated CCNU and meCCNU give indication of being more highly carcinostatic.

Figure 6 shows the urinary metabolites of CCNU 24 hours following iv injection of ^{14}C-chloroethyl-CCNU in the rat as reported by Reed and May (10). Over 60% of the radioactive dose appeared in the urine, with approximately half being identified as thiodiacetic acid and *S*-carboxylmethyl-1-cysteine. Thus, about 50% of the urinary ^{14}C can be accounted for by derivatives that are derived from thiol alkylation. It remains to be determined whether this thiol alkylation results from the 2-chloroethyl carbonium ion directly or from formation of 2-chloroethanol and 2-chloroacetaldehyde during the breakdown of the chloroethyl moiety of the nitrosoureas.

While metabolic studies are incomplete, they indicate that the pharmacokinetics of the nitrosoureas involve very rapid distribution and metabolites with different potential reactivities. In the case of CCNU and meCCNU, hydroxylation occurs rapidly enough to allow metabolism of large enough portions of the doses before chemical decomposition occurs. What effect these more polar metabolites have on carbamoylating function and rates of formation of alkylating species remains to be determined.

To summarize, pharmacologic disposition studies of BCNU, CCNU, and meCCNU indicate very rapid absorption, distribution, and metabolism which is mediated in part by microsomal enzymes. Peak plasma levels of a combination of metabolites and degradation products appear within 1–6 hours following oral drug administration in man. The plasma levels of the products are prolonged but they are primarily excreted in the urine. In CCNU, the isola-

[3]Reed DJ. Personal communication.

[4]1-Methyl-1-nitrosourea: NSC-23909; CAS reg. No. 684-93-5.

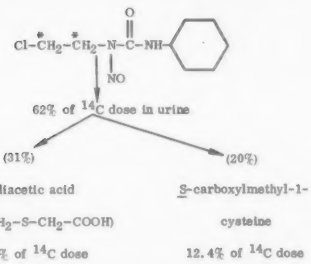
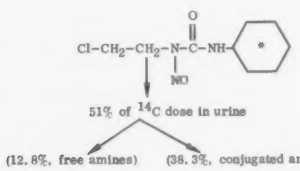


FIGURE 6.—Rat 24-hr urinary metabolites of ^{14}C-chloroethyl-CCNU (*see* ref 10).

TABLE 4.—Rat 24-hr urinary metabolites of ^{14}C-cyclohexyl-CCNU*



	% of dose excreted as—	
	Free cyclohexylamine bases	Conjugated cyclohexylamine bases
trans-3-hydroxy	1.1	1.8
cis-3-hydroxy	3.2	3.2
cis-4-hydroxy	2.5	2.0
trans-4-hydroxy	1.2	1.6
Total	8.0	8.6

*See footnote 3.

tion of free and conjugated hydroxylated cyclohexylamines, thiodiacetic acid, and *S*-carboxylmethyl-1-cysteine indicates the nature of the fate of both the 2-chloroethyl and cyclohexyl moieties of the molecule. It must be better understood how these metabolites and their postulated reactive intermediates relate to differentiation between alkylation and carbamoylation reactions as a basis for elucidating the mechanisms by which nitrosoureas exert their toxic and antitumor effects.

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The Nitrosoureas: An Outline of Clinical Schedules and Toxic Effects

Todd H. Wasserman[1,*]

As discussed in other papers presented in this issue, the nitrosoureas (BCNU, CCNU, and methyl-CCNU) (1–3)[2] are a group of rationally synthesized anticancer agents. Initial preclinical antitumor activity was demonstrated for this class of compounds in 1958 under a National Cancer Institute contract, and development of analogs led to the clinical trials of the three nitrosoureas discussed in this and other papers.

In 1962 the initial phase I trial of BCNU (4) yielded clinical activity in lymphomas; further analog development continued, with CCNU and methyl-CCNU subsequently entering clinical trial in 1969 (5) and 1971 (6) respectively.

The preclinical rationales for the clinical trial of CCNU were (*a*) its superior activity to BCNU in the intracerebral L1210 leukemia mouse tumor, (*b*) its asymmetric molecule allowing easier radiolabeling and pharmacokinetic studies, and (*c*) its oral clinical formulation. The preclinical rationale for the clinical trial of methyl-CCNU was its highly superior activity in the Lewis lung carcinoma mouse tumor, a tumor with kinetics allegedly predictive for human solid tumors (7).

The nitrosoureas as a drug class have a spectrum of useful clinical activity as discussed in other papers in this issue. Comparisons among the different nitrosoureas regarding clinical efficacy are difficult to make because of deficiencies in the clinical data from a comparative viewpoint (8). The clinical use of the nitrosoureas continues to be investigational in less common tumor types.

Dose Schedules

Phase I–II studies have elucidated the common dose schedules given in table 1. The names given for each drug are not trade names but are designations for generic names approved by the US Adopted Names (USAN) Council. The intermittent (q6–8wks) dose schedule was developed because of the delayed dose-limiting myelotoxicity. The range of the dose is dependent on the extent of prior therapy and the status of the bone-marrow reserve. Single doses of BCNU are fractionated to diminish local and/or gastrointestinal toxicity. CCNU and methyl-CCNU are usually given as single doses. The clinical use of other dose schedules or formulations is being investigated.

The qualities of the nitrosoureas (intermittent single-dose schedule, oral administration, no unusual clinical toxicities, and varied spectrum of clinical activity) have made it desirable to test them in combination with other antineoplastic agents, such as alkylating agents, antimetabolites, antitumor antibiotics, and other synthetics in many two-, three-, four-, and five-drug combinations. The doses of the nitrosoureas when used in combination generally tend to be 50%–75% of the single-agent dose. There have been no unusual antagonistic effects of the nitrosoureas with other anticancer drugs regarding either toxicity or efficacy. The clinical use of the nitrosoureas in combination continues to be investigational regarding the efficacy of such combinations.

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[2]BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-.

CCNU: NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-.

Methyl-CCNU: NSC-95441; CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-.

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TABLE 1.—Clinical dose schedules of the nitrosoureas

Nitrosourea	USAN name	Dose (mg/m²) and schedule	Dose formulation
BCNU	Carmustine	200–225, iv q6–8wks or 80, daily × 3 iv q6–8wks	100-mg vials (iv use only)
CCNU	Lomustine	100–130, orally q6–8wks	10-, 40-, and 100-mg capsules
Methyl-CCNU	Semustine	200–225, orally q6–8wks	10-, 50-, and 100-mg capsules

Toxicity Data

The phase I–II studies have also elucidated the clinical toxicity. Toxic effects of the nitrosoureas primarily involve the gastrointestinal tract and bone marrow. The three nitrosoureas produce similar qualitative toxic effects in animals and in man. Quantitatively, no real toxicity differences are discernible among the three drugs.

The gastrointestinal toxicity consists of acute nausea and vomiting (2–6 hrs after a dose and persisting < 24 hrs), not usually dose limiting and apparently centrally mediated (so that absorption of the oral drug has preceded the toxicity). The nausea and vomiting may be reduced by administering antiemetics or by fasting.

The bone-marrow toxicity consists of: (*a*) thrombocytopenia—occurring about 4 weeks after a dose with a median nadir of 80,000–100,000 platelets/mm^3 of 1–2 weeks' duration; and (*b*) leukopenia—occurring about 6 weeks after a dose with a median nadir of 4000–5000 cells/mm^3 of 1–2 weeks' duration. The bone-marrow toxicity is delayed, dose related, dose limiting, and cumulative. The cumulative effects are manifested by lower nadirs at a given dose level, earlier appearance of nadirs, longer durations of myelosuppression, or average nadirs at a lower dose level. In general, the severity of the thrombocytopenia forewarns for similar, but frequently less severe, ensuing leukopenia.

Prior radiotherapy and/or chemotherapy and/or bone-marrow infiltration with cancer will increase the bone-marrow toxicity, and lower initial doses should be considered for such patients. Blood counts should be observed prior to each dose of drug and weekly for the 3rd to the 6th weeks between doses for each of the first two to three doses. Dose schedules should be adjusted (by decreasing the dose or increasing the time interval between doses) based on both nadir and recovery blood counts prior to the next dose of the nitrosourea. A repeat dose may be given when blood counts return to acceptable levels but never before documented recovery from significant nadirs (not more frequently than q6wks).

The gastrointestinal and bone-marrow toxicities are to be expected in every patient. These toxicities are also those associated with most cytotoxic, anticancer drugs which affect proliferating cells.

Other infrequent toxic effects are stomatitis, alopecia, mild anemia, anorexia, elevations of liver function tests, and possible neuro-opthalmologic effects. In addition, BCNU may cause burning, hyperemia, and occasional phlebitis at the injection site. BCNU also causes occasional conjunctival flushing, elevated blood urea nitrogen levels, and possible pulmonary toxicity. Therefore, liver and kidney function should be monitored periodically.

Because of the above potential toxic effects, patients should be closely followed by a physician experienced in cancer chemotherapy. Also, treatment should occur within reach of facilities which have platelet transfusions available.

Pharmaceutic Data

BCNU is available in 100-mg single-dose vials for iv use only (no preservatives). The BCNU powder is reconstituted into solution (10% alcohol) according to the package instructions. This solution may be administered as is, slowly, or further diluted with sodium chloride or 5% dextrose for injection (USP). Unopened vials of the dry powder must be stored in a refrigerator (2°C–8°C). This recommended storage of unopened vials prevents significant decomposition for at least 2 years. Normal room temperature storage (22°C) of the unopened vials will result in a slow decomposition of the drug (approximately 3%) in 36 days. After reconstitution as recommended, BCNU decomposition at room temperature is linear with time. Approximately 6% of the solution decomposes after 3 hours and approximately 8% decomposes after 6 hours. Therefore, the reconstituted solution should be used as soon as possible.

CCNU is available in 10-, 40-, and 100-mg capsules and methyl-CCNU is available in 10-, 50-, and 100-mg capsules. The storage of unopened bottles in the refrigerator will assure stability for at least 2 years. Opened and partially used bottles of capsules are stable for a long period of time provided excessive moisture and heat are avoided.

In summary, the nitrosoureas are a new class of synthetic anticancer compounds with efficacy in a varied spectrum of tumor types. They have the usual toxic effects, gastrointestinal toxicity and myelosuppression, with myelosuppression being uniquely delayed. They have no other major organ toxicity. They are capable of being given orally on convenient intermittent schedules. Their use in clinical cancer therapy has been and will continue to be significant either as single agents or as part of combination chemotherapy.

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Clinical Studies in Malignant Gliomas and Their Treatment With the Nitrosoureas

Michael D. Walker[1,*] and Edmund A. Gehan[2]

Natural History and Prognostic Factors

Physicians have been aware of brain malignancies since the first descriptions of cancer, yet the true population incidence (in contradistinction to autopsy series) has been generated only within the last few years. The best available data indicate an age-adjusted incidence rate of 4.5 cases per 100,000, with men affected slightly more frequently than women (1). The disease, by its very nature, is usually localized with an unusually low rate of metastasis. Anaplastic gliomas in adults almost never metastasize while medulloblastomas and, less frequently, ependymomas in children have a greater tendency to spread beyond the confines of the central nervous system (CNS). The age distribution of brain tumor is biphasic with an early peak seen between the ages of 6 and 9 years (predominantly astrocytomas of the cerebellum, medulloblastomas, and ependymomas) and a second peak between the ages of 40 and 60 years (primarily malignant gliomas, meningiomas, and adenomas). Between 8000 and 9000 patients will die from brain malignancy during 1976 (1). Therefore, compared with other cancers, brain malignancy is not commonly found. It is seen, however, more frequently than Hodgkin's disease and about half as often as leukemia, both of which are disease entities in which we have had considerable success by use of a variety of treatment modalities.

"Brain tumor" is a term indicating a neoplasm within a specific location, rather than describing a specific biologic or histologic disease. In order to place malignancies within the CNS in some reasonable perspective, we must define more precisely the neoplasm of interest. The approximate frequency of occurrence of various brain tumors is shown in table 1 (2). One of the more important phenomena which, in all probability, is reported artifactually low in table 1 is the occurrence of metastatic disease to the brain. As control of cancer elsewhere becomes a greater reality and the patients live longer, it is quite likely that we will see an increasing incidence of spreading of these neoplasms to the brain. Considering that 20% of the cardiac output is directed to the brain, the reasons for this become more apparent. It is possible that we will reach a "second plateau" in the control of cancer which will be forewarned by the spread of the cancer to the CNS.

TABLE 1.—Incidence of different brain tumor types (*adapted from* ref 2)*

Type	% of brain tumors
Glioma	43
Glioblastoma multiforme	23
Astrocytoma	13
Ependymoma	1.8
Oligodendroglioma	1.6
Mixed and other	1.9
Medulloblastoma	1.5
Meningioma	16
Pituitary adenoma	8.2
Neurilemoma	5.7
Craniopharyngioma	2.8
Sarcoma	2.5
Hemangioblastoma	2.7
Pineal tumor	1.1
Metastatic	13.0
Other	6.0

*17,580 brain tumors studied.

It is clear that the most common tumors are those which are classified as malignant gliomas and contain the highly malignant and completely lethal glioblastoma multiforme. For the purposes of this paper, and unless otherwise specified, all references will pertain to malignant gliomas. Although there are other neoplasms associated with brain cancer, such as breast cancer with meningioma and gliomas with leukemia or phakomatosis, there is no indication of a true etiologic factor producing brain cancer.

A malignant glioma of the brain is always lethal (fig 1). The composite survival curve derived from many cases within the literature and normalized

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around common parameters for the purposes of statistical analysis, indicates the median survival of this disease to be approximately 23 weeks. At the end of 1 year 20% of the patients are alive and after 2 years almost 90% have died from their disease. It is clear, then, that vigorous and active therapy should be pursued in the treatment of this disease.

The profile of a patient with a malignant glioma is relatively consistent, although the precise details may vary (3). The patient may be of either sex and is usually between 40 and 60 years of age. The history is frequently developed retrospectively, with signs starting about 6 months before diagnosis. At that time, the patient may develop occasional headaches and undergo subtle personality changes, the extent of which do not become apparent until later. It is only during retrospective discussions with the patient and his family or co-workers that a consistency to the history may be found. Motor symptoms may be present but are frequently denied, and on relatively few occasions seizures may herald the onset of the disease. These generalized symptoms are usually attributed to current events in the lifestyle of the patient, increasing work demands and pressures, or accidental events. It is not until the symptoms show signs of progressing or there is a convulsion or accident that medical attention is sought. All of these events may be telescoped to within a matter of a few hours by sudden precipitous events, but even then, a careful, probing history will frequently elicit early subtle changes.

Once the patient is examined a diagnosis is usually established quickly. At the time of admission, approximately three quarters of the patients with malignant gliomas have motor signs which may or may not be of localizing value. Almost two thirds of the patients will have mild to moderate personality changes and almost one half will have an altered level of consciousness or awareness (3).

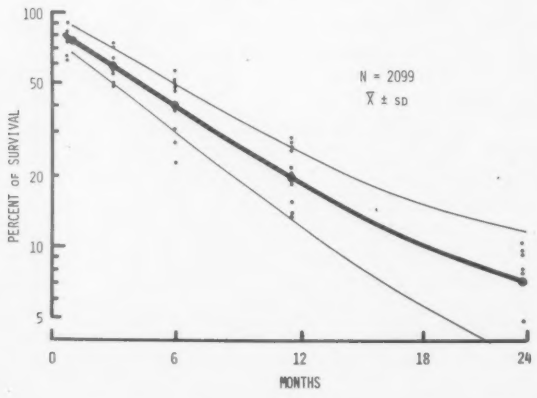

FIGURE 1.—Percent of malignant-glioma patients surviving after surgery.

A series of diagnostic examinations, including neurologic tests, radionucleotide brain scanning, computerized axial tomography scans, and arteriography, will establish the presence of a space-occupying lesion, its relationship with contiguous brain, and abnormal or anatomically displaced vasculature. An appraisal of the anticipated pathologic diagnosis may be made on the basis of neurodiagnostic findings. During surgery, the surgeon will note specific characteristics of the tumor, including its location, degree of vascularity, presence of necrosis, encapsulation, or cyst formation. Despite attempts at vigorous therapy, he will be unable to entirely excise the tumor, although all gross tumor visible may be removed. Despite this apparent shortcoming, the surgeon has been able to accomplish a number of definitive things. He has reduced the tumor mass by an order of magnitude which no known adjuvant therapy today can do so quickly and safely. He has reduced the tumor burden within the closed cranial vault and, thus, provided the time required to institute adjuvant therapy. The tissue removed will allow the establishment of a histologic diagnosis which may be compared to both therapeutic approaches, as well as changes in histology over the course of time. From the various symptoms and sign complexes noted, the patient population characteristics, and the operative findings, a large variety of data may be generated which may be studied utilizing relatively sophisticated techniques.

To more clearly define various parameters of this disease entity, the Brain Tumor Study Group (BTSG),[3] a cooperating group of neuro-oncologists, and the Division of Cancer Treatment have undertaken the collection of a wide variety of data elements. These elements include patient characteristics, symptom complexes and timing, and tumor characteristics:

Patient characteristics.—(*a*) Age, (*b*) sex, (*c*) tumor location (right or left [frontal, temporal, parietal, occipital, central, cerebellum]), and (*d*) duration of symptoms.

Symptom characteristics.—Headache, personality change, motor symptoms, seizures, speech disorder,

[3]The following institutions are the current participants of the BTSG: Bowman Gray School of Medicine; Indiana University Medical Center; Ohio State University Hospital; Duke University Medical Center; New York University Medical Center; Sloan-Kettering Memorial Hospital; University of Kentucky Medical Center; St. Louis School of Medicine; University of California Medical Center, San Francisco; University of Tennessee; University of Pittsburgh-Montefiore Hospital; University of California, Los Angeles; Universita de Pavia; and BCRC.

sensory symptoms, cranial nerves (II, III, IV, VI), rapid unconsciousness, cranial or other nerves, cerebellar symptoms, general systemic complaints, and other.

Tumor characteristics.—Extent of resection, hypovascular, invasive, solid, necrotic, soft, friable, cystic, hard, vascular, and encapsulated.

These data are currently under analysis to determine those disease characteristics which appear to correlate negatively or positively with survival. The analysis is a two-step procedure in which individual characteristics are first related to survival to determine if there is any indication of a possible correlation. Characteristics which appear to have some relationship to the prognosis are then entered into a stepwise regression analysis using Cox's model fit to the survival data (4,5). A more detailed description of this procedure and its results is being prepared.[4]

A first approximation indicates that four of the characteristics examined appear to correlate positively with survival, that is, the presence of these findings predicts a longer survival (or a lower hazard function). These characteristics include: (*a*) a long interval between the first symptom and surgery; (*b*) the presence of seizures in the symptom complex; (*c*) involvement of cranial nerves II, III, IV, and VI in the symptom complex; and (*d*) a description by the surgeon at the time of surgery of encapsulation of the tumor. Three specific characteristics appear to correlate negatively with the chances for an increased survival. They include (*a*) increasing age, (*b*) a parietal or central location of the tumor, and (*c*) an operative procedure which is restricted to biopsy only.

While this only represents a preliminary analysis, the application of these prognostic factors will provide a better understanding of the biologic variables present in dealing with malignant gliomas. It will also permit more refined analysis of data as they are generated in the future. Because they are important characteristics that relate to survival, they should be included in comparability analyses of various therapeutic arms to ensure that the respective treatment groups have been drawn from a similar population, and that survival is not being influenced by nontherapeutic factors. The method of using this information and applying it will await confirmation of the qualitative estimates noted above and their translation into quantitative data.

Therapeutic Studies With the Nitrosoureas

The BTSG has undertaken two prospective, controlled, and randomized studies in the treatment of malignant gliomas using the nitrosoureas. Each study conducted by the BTSG performs two major tasks. First, an attempt is made to identify information basic to an understanding of the disease entity. Second, specific modes of therapy are evaluated in a controlled fashion. The first BTSG study (3) established that anaplastic glioma could be evaluated in controlled, prospective, and randomized studies similar to those designed for the study of other diseases. It further indicated that mithramycin was of no value in the treatment of this disease. The second study was designed to quantitatively define the efficacy of radiotherapy (as opposed to qualitative estimates) and to indicate the role which it plays in the treatment of malignant gliomas. From a chemotherapeutic point of view, the study evaluated the value of BCNU[5] alone and in combination with radiotherapy as a potentially effective mode of treatment. In uncontrolled phase II studies, Walker et al (6) and Wilson et al (7) indicated that one half of a series of patients with malignant gliomas treated with BCNU appeared to have a clinical response of varying duration.

The BTSG, therefore, designed a controlled study in which patients, within 3 weeks of definitive surgical resection and after establishing the diagnosis of glioblastoma, were randomized to receive BCNU (80 mg/m^2/day on 3 successive days every 6–8 weeks), radiotherapy (5000–6000 rads over 6–8 weeks), a combination of both BCNU and radiotherapy in the doses mentioned above, or a continuation of the best conventional care. Two hundred and twenty-five of 303 patients entered in the study satisfied the protocol requirements and were therefore considered in the *valid study group.* The median survival of those who received the best conventional care, but no additional chemotherapy or radiotherapy, was 14.0 weeks, while those who received at least one dose of BCNU had a median survival of 18.5 weeks. Patients who received radiotherapy (not necessarily the full prescribed dose) had a median survival of 36.0 weeks, whereas those who received some BCNU and radiotherapy had a median survival of 34.5 weeks.

Since not all patients received optimal doses of treatment, it was decided to establish the *adequately treated group,* a subset of the *valid study group.* In order to be included in the *adequately treated group,* all patients assigned to receive radiotherapy must have received ≥ 5000 rads, those treated with BCNU must have received two or more courses of treatment, and all patients must have

[4]Gehan EA. Prognostic factors for patients with brain tumors. In preparation.

[5]BCNU: NSC-409962; CAS reg. No. 154–93–8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-.

had a minimum guaranteed survival of 8 weeks, ie, the amount of time required to receive the above prescribed radiotherapy and/or chemotherapy. An analysis of this group of patients indicates a median survival of 17.0 weeks (best conventional care), 25.0 weeks (BCNU), 37.5 weeks (radiotherapy), and 40.5 weeks (BCNU plus radiotherapy). Statistical analysis by the Wilcoxon test indicates that all forms of therapy are significantly better ($P = <0.003$) than no therapy at all. Although radiotherapy and BCNU appear to be better than radiotherapy alone, the difference is not statistically significant. At 18 months virtually all of the patients receiving the best conventional care or BCNU had died from their disease, while 9% of those who received radiotherapy and 18% of those receiving combination therapy were still alive.

This study has indicated that BCNU and radiotherapy in adequate amounts are both effective in the treatment of malignant glioma. The combination of BCNU and radiotherapy is only marginally better than radiotherapy alone. The toxicity of BCNU is at an acceptable level and serious thrombocytopenia is not frequently seen. Based upon the results of this study, it was felt that further studies were warranted.

Methyl-CCNU,[6] the newest of the nitrosoureas to be used in a clinical trial, was chosen as the new drug for evaluation. A protocol with four treatment arms was designed in which methyl-CCNU (220 mg/m^2 orally every 6–8 weeks), radiotherapy (6000 rads to the whole head through bilateral opposing ports over 6–8 weeks), methyl-CCNU and radiotherapy (in the above dosages), and the best arm of the previous study (BCNU and radiotherapy) were compared. This study is no longer accepting patients; however, it is still continuing and therefore these results are only preliminary. Three hundred and forty-nine patients have been randomized to the four treatment arms. There is sufficient information available on 253 of these patients for a preliminary analysis. Patients receiving methyl-CCNU alone had a median survival of 31 weeks while those who received radiotherapy had a median survival of 36 weeks. Those who received a combination of methyl-CCNU and radiotherapy had a median survival of 34.6 weeks while those who received BCNU and radiotherapy had a median survival of 50.3 weeks. An analysis of comparability factors, including age, sex, duration of symptoms, time interval from surgery to randomization, and amount of therapy received, indicates that there is no significant difference between these four subpopulations. It would be premature to draw therapeutic conclusions from these data until all of the data are in and the definitive analysis can be performed. However, the previous findings of the efficacy of BCNU and radiotherapy appear to be substantiated.

The nitrosoureas, particularly BCNU and methyl-CCNU, have been carefully studied in controlled evaluations. These drugs are the first to show modest but significant value in the treatment of malignant brain tumors. Their relationship with the utilization of radiotherapy is not entirely clear. It may well be that their usefulness is more obvious in recurrent glioma, as the preliminary results utilizing CCNU have indicated (8). The ability to show increased survival from the use of the nitrosoureas is the first step which points the way toward the design of more definitive studies which need to be performed from a clinical, pharmacologic, and multitherapeutic standpoint.

[6]Methyl-CCNU: NSC-95441; CAS reg. No. 33073–59–5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-.

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Role of Surgery in the Treatment of Malignant Gliomas[1]

Joseph Ransohoff[2,*]

The role of intracranial surgery in the treatment of malignant gliomas must be justified on the basis of good surgical principles as well as on the principles of sound cancer therapy. Surgery, by itself, will never cure glioblastomas or grade III or IV malignant gliomas. Formal intracranial surgery is not necessary in order to establish the diagnosis since this can often be made on the basis of patient history, appearance of abnormal vascularity on cerebral angiography, and appearance of the tumor on radioactive brain and computerized transaxial tomography (CTT) scanning. A simple needle biopsy through a tiny burr hole can be performed with minimal risk if a definitive tissue diagnosis is desired. From the point of view of investigators interested in cancer treatment, formal intracranial surgery is justified in order to remove the largest part of the tumor, thereby reducing the number of malignant cells to be exposed to radiation therapy, chemotherapy, immunotherapy, or whatever mode of therapy is directed towards the preservation of life and the destruction of the tumor. In this particular cancer where rapidly growing invasive cells are at the tumor margin and where the center is relatively avascular and at times necrotic, the removal of this large central tumor is even further justified because it is within this area that the remaining viable cells exist in a relatively indolent fashion. These cells are the hardest to reach with chemotherapeutic agents and are incriminated by many investigators as the cells which account for the continued relentless tumor growth.

The demands of successful intracranial surgery must meet the criteria of low mortality and morbidity, and at the same time the removal of a large percentage of the tumor cells. To achieve these goals, careful planning must be carried out before intracranial surgery, since "wounding" or only partially removing these tumors often leads to considerable swelling of the tumor itself and the surrounding brain with poor postoperative results. The routine pre-operative evaluation includes the use of brain scanning and cerebral angiography and more recently the use of CTT scanning, which is extremely useful in the planning of radical surgery. The CTT scan clearly shows the relationship of the tumor to the ventricular system and will often show that the largest part of the tumor is restricted to the central white matter, displacing the ventricular system. On the other hand, the same tumor on brain scan and angiography would be interpreted as being present within structures such as the internal capsule and deep central gray masses which, if violated at the time of surgery, would lead to irreversible paralysis and/or coma. On the basis of these principles, radical intratumor surgery can be performed in almost every intracranial location with the following exceptions: tumors within the deep central areas of the dominant hemisphere, the extensive posterior bilateral corpus callosum tumors, and those located within the brain stem itself. The use of large doses of corticosteroids before surgery as well as during the postoperative period supplemented by intra-operative osmotic diuretics will prevent disastrous brain swelling during surgery and allow for the replacement of the bone flap. Discarding of the bone flap should not be considered in the treatment of these patients except in the most unexpected situations since it generally leads to severe morbidity after surgery and to the eventual development of a cerebral fungus; discarding of the bone flap is usually a sign of a poorly planned surgical procedure. Surgery should, therefore, not be undertaken simply to confirm the diagnosis and should only be used when there is a reasonable chance of achieving the above-outlined objectives.

I have reviewed the results of 30 consecutive patients with glioblastomas who had surgery in the past 2 years. There were 14 women and 16 men in the group with an average age of 56 years (range, 21–73 years). There were 18 right- and 12 left-sided tumors in this series confirming the well-known fact that deep tumors in the center of the dominant hemisphere are not amenable to radical surgical removal. The locations of these 30 tumors are shown in table 1.

In order to evaluate the results of radical surgical removal, these patients were rated according to the Karnofsky scale which is undoubtedly the most

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TABLE 1.—Grade IV gliomas

	Location	
	Right	Left
Frontal	1	4
Frontotemporal	6	4
Tempoparietal	4	1
Parieto-occipital	2	2
Frontoparietal	2	1
Holohemispheric	3	0
Totals	18	12

TABLE 2.—Maximum Karnofsky scale rating achieved 2 mos after radical surgery (1973–1975)*

Rating	No. of patients†	No. of patients with tumors on the left side
100	4	1
90	12	5
80	3	2
70	2	1
60	4	2
50	1	1
40	1	1
≤ 30	3*	

*1 death occurred during the first 2 mos after surgery. All patients also received radiation therapy or chemotherapy plus radiation therapy.

†19 of 30 patients had a rating of ≥ 80.

valuable system of grading the results of treatment of central nervous system disease since it is based on the patient's ability to carry out the functions of daily living. The Karnofsky scale ratings (in abbreviated form) are as follows: 100 = normal; 90 = normal, minor symptoms; 80 = normal activity with effort; 70 = self care; 60 = occasional assistance; 50 = frequent care; 40 = disabled; 30 = severely disabled; 20 = very sick; and 10 = moribund. It is important to note that patients with occipital tumors could not achieve a rating > 80 because a postoperative hemianopic defect automatically placed them in this category even if they were normal in every other aspect. Table 2 shows the maximum Karnofsky scale rating achieved by these 30 patients 2 months after surgery was performed. Nineteen of the 30 patients achieved a Karnofsky scale rating of ≥ 80. There was one operative death during the first 2 months after surgery in a patient whose glioblastoma recurred in a dramatic fashion in spite of radical surgical removal, radiation therapy, and chemotherapy. After surgery all the patients received radiation therapy and several received chemotherapy as based on the Brain Tumor Study Group protocol.

One factor added to the diagnostic procedures available at our Medical Center during the 2 years mentioned above was the CTT scan. The value of this diagnostic method presumably has partially accounted for the difference in the Karnofsky ratings when the 1973–1975 patients were compared with those patients who had surgery in the prior 2 years. Only ten of 27 patients or a little more than one third achieved a Karnofsky rating of ≥ 80 in the 1971–1973 group whereas almost two thirds achieved this rating in the subsequent 2 years when the CTT scanner was available.

CONCLUSIONS

No attempt has been made to establish the value of radical surgery in the treatment of malignant gliomas in this brief paper. I have, however, attempted to document the fact that a reasonable number of patients can undergo an energetic surgical attack on their tumor with a reasonable expectation for good short-term recovery. This paper, therefore, has been designed merely to demonstrate the capabilities of modern surgical techniques in a total treatment program for patients with malignant brain tumors.

Nitrosourea Chemotherapy for Primary Malignant Gliomas[1]

V. A. Levin[2,*] and C. B. Wilson[3,4]

To optimize therapy for malignant gliomas, it is necessary to take into account some of the special characteristics of primary brain tumors. Although malignant gliomas are similar to other solid tumors in terms of their relatively high neoplastic cell densities, low-growth fractions, edematous regions, and abnormal vessels, they differ in important ways. Malignant gliomas are highly infiltrative tumors which reside in an irreplaceable organ, and there is little room for asymptomatic tumor expansion within the brain and nonexpansive dural and osseous cranial vault.

Tumor and Brain Adjacent to Tumor Milieu

Some of the characteristics of the tumor milieu which are important "local" features of malignant gliomas are:

a. neoplastic tissue with high cell density;
b. infiltrative primary tumors;
c. edematous regions and sequestered cell populations;
d. abnormal "leakiness" of vessels secondary to gaps, fenestrations, etc, proportional to degree of malignancy;
e. abnormal cell permeability, probably proportional to degree of malignancy; and
f. tumor located within rigid dural and osseous cranial vault.

Of equal if not greater importance are the characteristics of the brain adjacent to tumors (BAT) which are:

a. edematous regions generally involving white rather than gray matter;
b. infiltrative cells from primary tumors;
c. vasculature frequently appears normal; and
d. nearly 50% reduction in capillary permeability coefficient to water-soluble compounds.

Because malignant gliomas are infiltrative tumors, tumor cells frequently migrate 1–2 cm from the main tumor and ahead of histologically abnormal vessels. Some recent evidence suggests that certain capillaries in the BAT may be even less permeable to water-soluble drugs than normal brain capillaries (1). This does not hold true, however, for the lipophilic nitrosoureas; these drugs distribute equally in normal brain, BAT, and tumor (2).

Theoretic and Experimental Considerations for Nitrosourea Therapy

The nitrosoureas show steep dose-response (ie, cell kill) curves by cell culture survival studies in vitro and by colony-forming efficiency assays in vivo (3,4). Similarly, repopulation cell kinetics are dose dependent and the half-life for repopulation may differ > 2.5-fold for dose differences of 25%–100% of the LD10. This has been observed in the case of BCNU for the intracerebral 9L gliosarcoma in the rat (5) and also for other nitrosoureas in different tumor systems. These observations indicate that because of the steepness of the dose-response curve for the nitrosoureas, effective therapy will require high-dose schedules at longer intervals of time rather than lower-dose schedules at shorter intervals.

Recently, Rosenblum et al have found experimental evidence suggesting that single high doses of BCNU are as effective as smaller consecutive doses that add up to the same total dose (5). This would have practical ramifications for BCNU chemotherapy regimens because a common schedule (*see* below) has been 80 mg/m²/day × 3. Replacing this schedule with a 1-day total dose of 200–220 mg/m² would result in 2 fewer treatment days. Although more immediate toxicity (nausea and vomiting) might be expected with single large doses of BCNU, consideration should be given to single injections of BCNU.

While the steepness of the dose-response curve and the response to fractionation of the BCNU dose may be common to all solid tumors, the seriousness

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of tumor enlargement is unique to brain tumors. The brain resides within a closed "box," the skull. Any expansion of intracranial contents, even to the extent of a 1%–2% increase in volume, might, if abrupt, cause immediate transtentorial herniation or, at the least, obtundation and exaggeration of pre-existing focal neurologic signs. There are three ways in which this expansion could occur as a result of nitrosourea therapy whether or not it is "effective." (*a*) There may be intracellular "swelling" and increased tumoral edema if membrane or intracellular damage is severe enough to cause osmotic shifts of water. (*b*) Some of the very immediate antitumor effects of the nitrosoureas may theoretically cause these changes days to weeks later because of an increase in tumor volume as a result of cell division following genetic damage in tumor cells. Frequently, tumor cells effectively treated with a nitrosourea will undergo two to three cell divisions before they "die."[5] (*c*) There have been observations at biopsy (6) and under experimental conditions (6)[5] which suggest an increase in giant-cell formation with BCNU therapy. Any of the above factors can increase tumor-cell volume by a small factor which, because of the skull's rigidity, could become a serious threat to the patient. Compounding this problem further is the slowness of dead-cell removal in the brain compared to other tissues (8). Theoretically, these problems should not be life threatening following surgical decompression; their importance increases, however, with continued treatment in the absence of surgical intervention.

The last consideration, that of possible drug-drug interactions, is at present only a theoretic complication since no data exist to implicate secondary drug enhancement or diminution of nitrosourea metabolism in vivo. However, we should recognize that such interactions are inevitable and almost certainly will be detected as research progresses in this area.

Response Criteria and Methods of Therapy Evaluation

To evaluate patients undergoing brain-tumor chemotherapy, it is important to develop a methodic system for following patient response. Over the years we have found that a careful neurologic examination and isotope brain scan obtained at 4–8-week intervals (depending on therapy) will accurately predict response and deterioration to therapy. If the neurologic examination and isotope scan are obtained at the same time and one of the tests is positive (ie, better or worse) and the other is unchanged, response and deterioration to therapy can be accurately determined in 92%–96% of the patients.[6] The advent of the computerized axial tomogram (CAT) is changing our reliance on the isotope scan, but in sequential followup of 100 patients receiving chemotherapy in our service there appeared to be little advantage of the CAT over the isotope scan. Other benefits of the CAT scan make it a valuable neurodiagnostic addition, however, because complicating conditions such as hydrocephalus, intracerebral and intratumoral bleeding, porencephalic cysts, and intracerebral hemispheral shifts are now detectable, and would previously have been missed by conventional isotope scan techniques.

A caveat to the above considerations is that when glucocorticoids are used neurologic and isotope scan improvement may occur independent of cytotoxic drug therapy. Erroneous therapeutic conclusions can be avoided if the following is kept in mind: (*a*) steroid doses should be maintained at the lowest levels consistent with a useful and comfortable life for the patient; (*b*) if "clinical improvement" occurs, try lowering steroid doses to ensure that response to cytotoxic therapy is bonafide; and (*c*) escalation of steroid dose accompanied by clinical deterioration usually indicates deterioration due to tumor regrowth.

A further consideration in evaluating response to chemotherapy during or after radiotherapy is that some patients show clinical neurologic deterioration up to 3 months after radiotherapy has been discontinued. The reasons for this are not completely known, but during this period the response to steroids is dramatic. Usually the patient will require steroids for only a short period of time (1–2 weeks), reinforcing the conclusion that such deterioration is not a function of tumor regrowth but probably due to brain edema, slow dead-cell removal, etc. Within 3 months of radiotherapy some patients will be clearly deteriorating because of tumor regrowth and will be in need of additional therapy. However, care is required to distinguish these cases from patients showing post-irradiation deterioration.

Results of Chemotherapy With Nitrosoureas for Recurrent Malignant Gliomas

Table 1 summarizes the results of chemotherapy with BCNU and CCNU for recurrent and unbiopsied malignant gliomas. In general, patients with

[5]Wheeler KW. Personal communication, 1975.

[6]Levin VA, Norman D, Hoffer P, et al. The use of the neurological exam, isotope scan, CT scan, and EEG in evaluating response and deterioration to brain tumor therapy. In preparation, 1976.

TABLE 1.—Results of treatment with nitrosoureas for recurrent malignant gliomas between 1969 and 1975

Treatment	No. of responses*/ No. of evaluable patients	Median duration of response (mos)†	Ref No.
Single agents			
BCNU	22/43(51%)	9	12
CCNU	10/23(43%)	6	13
	3/8 (38%)	6	14
Drug combinations			
PCV	18/30(60%)	9	15
BCNU and procarbazine	21/45(47%)	7	16
BCNU and vincristine	7/17(41%)	4	17
CCNU, cyclophosphamide, and vincristine	3/8 (38%)	3	10

*Includes responders and probable responders. The CCNU, cyclophosphamide, and vincristine patients were all probable responders.

†Only for unequivocal responders; probable responders had median durations of response of 3–4 mos in all studies.

deep unbiopsied tumors have had malignant gliomas confirmed at autopsy. In the past 5 years we have been mistaken only twice in the diagnosis of malignant glioma from the clinical course and neurodiagnostic studies; in both cases the patients harbored reticulum cell sarcomas at autopsy.

Of interest also is that patients with deep or dominant hemisphere tumors who did not have surgical resections did almost as well as those who had resections, previous therapy, recurrence, and subsequent nitrosourea chemotherapy.

It is our belief from clinical experience and animal experimentation (9)[6] that BCNU is superior to CCNU when these drugs are used as single agents. However, for BCNU in combination with other agents, this conclusion may not be valid since a minor secondary cytotoxic function of one drug may be amplified by a second drug to make that particular combination of drugs superior.

In terms of duration of response, no therapy was superior to BCNU alone although the combination of procarbazine, CCNU, and vincristine (PCV) did have a 9% higher response rate (9). In the treatment of recurrent medulloblastoma we have found that this combination (PCV treatment No. 1) was extremely effective in initiating tumor regression. Table 2 summarizes the results of Crafts et al with this combination.[7] The major deterrent to the use of PCV No. 1 so far is the serious myelotoxicity observed. This was probably compounded by the fact that most of the patients had received prior radiotherapy to the spinal axis.

Our experience with BCNU therapy for recurrent ependymomas has been hard to evaluate objectively because most cases involved spinal cord disease, which does not lend itself to routine neurodiagnostic surveillance; clinical signs can fluctuate from visit to visit without dramatic change until a great deal of tumor regrowth has occurred. Even with these considerations we feel that BCNU is an effective form of palliative therapy for recurrent ependymomas. We have treated 12 patients and have been able to either maintain the status quo or achieve improvement in eight.

Table 1 also summarizes our results with recurrent malignant gliomas treated with a nitrosourea and one or two additional cytotoxic drugs.

Dose Regimens for Nitrosoureas as Single Agents

The use of the nitrosoureas as single agents in brain-tumor chemotherapy is clearly palliative. Al-

TABLE 2.—PCV combination therapy for recurrent medulloblastomas* (*from* footnote 7)

	No. of patients	Median duration of response (mos)
Evaluable	12	
Nonevaluable	1	
Responders	7 (58%)	10
Probable responders	4 (33%)	4
Nonresponders	1 (8%)	

*Age range of patients in this series was 5–51 yrs.

[7]Crafts DC, Wilson CB, Levin VA, et al. Chemotherapy of recurrent medulloblastoma with combined procarbazine, CCNU and vincristine. Submitted to *Cancer Treatment Reports.*

though the most successful therapy for malignant brain tumors will combine the best qualities of surgical resection (when feasible), radiotherapy, and combination chemotherapy, it is worthwhile to know typical dose regimens in order to plan more effective combination protocols.

Table 3 summarizes some of the regimens that we and others have used over the years. Because of the steepness of the dose-response curve for BCNU, the high-dose regimens which result in cumulative doses of 200–220 mg/m² every 8 weeks or 180 mg/m² every 6 weeks would seem preferable to doses of 120–130 mg/m² every 4 weeks. Similarly, CCNU at doses of 120–130 mg/m² every 6–8 weeks should be better than doses of 75 mg/m² every 4 weeks. In the case of BCNU our data support this viewpoint; CCNU has not been tested extensively enough for any definite conclusions to be drawn at this time.

Dose Regimens for Nitrosoureas in Combination With Other Agents

Table 4 summarizes some of the regimens for nitrosoureas used in combination therapy. BCNU and procarbazine therapy was used in the hope of achieving a better response than that previously obtained with PCV. This decision was based on the known effectiveness of BCNU and procarbazine, two of the most active agents in single-drug therapy for malignant gliomas (10). Disappointingly, the two drugs together were less active than BCNU alone. Severe myelotoxicity was a major problem with the BCNU and procarbazine combination. In addition, the reduced BCNU dose of 100 mg/m² probably represented an unnecessary compromise. It would be of value to look at a BCNU and procarbazine combination in which BCNU would be administered at a nearly full dose followed by a reduced dose of procarbazine.

BCNU and hydroxyurea combined with radiotherapy is a well-tolerated regimen originally developed by the Western Cancer Study Group as a phase III study for malignant gliomas. Results are not available at present to permit any conclusions regarding the superiority of this combination over BCNU alone plus radiotherapy.

A BCNU and 5-fluorouracil regimen was developed to determine the efficacy of cell-cycle-specific drug therapy at a time of maximum tumor repopulation after BCNU therapy. The regimen is well tolerated and myelotoxicity does not appear to be greater than that observed for therapy with BCNU alone. It is too early to decide whether this therapy will be more effective than BCNU used as a single agent for recurrent (phase II) malignant glioma patients.

The CCNU combinations are also listed in table 4. The original PCV protocol (PCV treatment No. 1) is shown. Although very effective for both recurrent malignant gliomas and medulloblastomas at the higher doses, it was quite myelotoxic and dose reductions were frequently associated with tumor regrowth. In an effort to reduce myelotoxicity PCV treatment No. 2 was developed following extensive studies with animals. If procarbazine therapy is begun 1 week after CCNU, and vincristine administration is timed to take advantage of maximal tumor cell repopulation, we anticipate that PCV treatment No. 2 will be superior to treatment No. 1. Preliminary data already indicate lower myelotoxicity.

CCNU and procarbazine (No. 3) is theoretic and thus far untested. It was conceived for treatment of malignant gliomas and metastatic tumors based on animal trials conducted in our laboratories. The CCNU is given at a relatively high dose and procarbazine is given 1 week later at a reduced dose.

In a pilot study, Taylor et al (11) evaluated radiotherapy in combination with methyl-CCNU or methyl-CCNU and DTIC for malignant gliomas. In the 13 patients studied they observed a median duration of survival of approximately 53 weeks. However, the series is too small to compare directly with the Brain Tumor Study Group studies or to draw any conclusions regarding the efficacy of methyl-CCNU compared to BCNU or CCNU.

TABLE 3.—Typical dose regimens for nitrosoureas

Drug	Dose (mg/m²)	Schedule
BCNU	80	× 3 days, q6–8wks
	200–220	× 1 day, q6–8wks
	120–130	× 1 day, q4wks
	180	× 1 day, q6wks
CCNU	120–130	× 1 day, q6–8wks
	75	× 1 day, q4wks
Methyl-CCNU	220	× 1 day, q6–8wks

Concluding Remarks

Today, the nitrosoureas are the most potent anticancer agents for malignant gliomas. Nevertheless, effective as they are they remain merely palliative forms of therapy. The future of brain tumor therapy requires that in addition to continuing our search for additional active agents, we maximize the potential strengths of the nitrosoureas by the careful design of drug combinations in which the nitrosoureas are the mainstays. In the design of these studies it will be wise to administer the nitrosoureas at relatively high doses at longer intervals as op-

TABLE 4.—Some combination drug protocols with nitrosoureas

Drug	Dose (mg/m²)	Schedule	Ref No.
BCNU combinations			
1. BCNU	100	Day 1, q6wks	16
+			
procarbazine	100	Days 2–21, q6wks	
2. BCNU	80	Days 1–3, q8wks	
+			
hydroxyurea	275	q6hrs, qod during radiotherapy	
+			
radiotherapy			
3. BCNU	180	Day 1, q6wks	
+			
5-fluorouracil*	1000	Infusion, Days 14–16, q6wks	
CCNU combinations			
1. PCV treatment No. 1			
CCNU	75	Day 1, q4wks	15
+			
procarbazine	100	Days 1–14, q4wks	
+			
vincristine	1.4	Days 1, 8, q4wks	
2. PCV treatment No. 2			
CCNU	75	Day 1, q6wks	
+			
procarbazine	100	Days 8–21, q6wks	
+			
vincristine	1.4	Days 8, 29, q6wks	
3. CCNU	100	Day 1, q6–8wks	
+			
procarbazine	75	Days 8–21, q6–8wks	
4. CCNU	130	Day 1, q6wks	11
+			
DTIC	175	Days 1–5, q4wks	
Methyl-CCNU combination			
1. Methyl-CCNU	175	Day 1, q6wks	11
+			
DTIC	175	Days 1–5, q4wks	

*5-FU was also repeated on Days 28–30 with acceptable toxicity.

posed to lower doses at shorter intervals. Because of the marked and cumulative marrow toxicity of the nitrosoureas, it will almost certainly prove beneficial to combine them with other agents of lesser or different toxicities. We eagerly anticipate the eventual discovery of such highly effective synergistic combinations.

LIST OF COMPOUNDS

BCNU: NSC-409962; CAS reg. No. 154–93–8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-

CCNU: NSC-79037; CAS reg. No. 13010–47–4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-

Cyclophosphamide: NSC-26271; CAS reg. No. 6055–19–2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate

DTIC: NSC-45388; CAS reg. No. 4342–03–4; imidazole-4-carboxamide, 5-(3,3-dimethyl-1-triazeno)-

5-Fluorouracil: NSC-19893; CAS reg. No. 51–21–8

Hydroxyurea: NSC-32065; CAS reg. No. 127–07–1

Methyl-CCNU: NSC-95441; CAS reg. No. 33073–59–5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-

Procarbazine: NSC-77213; CAS reg. No. 491–88–3; *p*-toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, monohydrochloride

Vincristine: NSC-67574; CAS reg. No. 2068–78–2; leurocristine, sulfate (1:1)

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Nitrosourea Pharmacodynamics in Relation to the Central Nervous System

Michael D. Walker* and John Hilton[1]

Although the nitrosoureas have been available and studied for over a decade, in-depth analysis of the pharmacokinetics, metabolism, and mode of action has been sparse. Little is known concerning the metabolic products of these substances; however, it is becoming clear that they are quite rapidly broken down once they are administered and may have a variety of intermediate products. The chemistry and mechanism of action have been described elsewhere in this issue by Bono and Montgomery (1,2). The human pharmacology has been more difficult to explain because of technology limits and the doses of the nitrosoureas which may be clinically administered. Although radiolabeled material has been available and has been utilized in man, the total dose which can be administered and the limits of sensitivity of analytic equipment have severely limited our ability to study these drugs in depth (3).

In considering the entrance of any drug into the central nervous system (CNS), the sequence of aqueous and lipophilic conditions through which a drug must pass unscathed must be understood (fig 1). After oral administration (as with CCNU and methyl-CCNU),[2] the drug must survive the altering pH and the chemical and enzymatic attacks which take place within the stomach and small bowel, and must cross the intestinal wall and survive hepatic degradation of the enterohepatic circulation. Once the drug is in the blood stream it may be metabolized, become bound to plasma protein, or become ionized. Upon its arrival at the CNS, it must be capable of traversing the capillary endothelium and tight junctions of the blood-brain barrier (a lipophilic phenomenon) and must enter the extracellular space of the brain (an aqueous medium). The agent must then cross the cellular membrane of tumor cells, enter cytoplasmic water, and finally arrive at the proposed area for its activity. It is remarkable that any drug can survive these various conditions.

The drugs which enter the CNS must possess the traditional characteristics required for permeability through the blood-brain barrier. Although they must be lipid soluble, new light has been shed on this phenomenon with the development of the log P concept, ie, the log of the octanol/water partition coefficient described by Hansch et al (4). It is becoming apparent that drugs may also be excessively lipid soluble or so aqueous insoluble as to be undeliverable under physiologic conditions. The log P of effective agents such as the barbiturates and narcotics ranges between two and three. Another characteristic relevant to the blood-brain barrier is a small molecular weight, with very free exchange taking place between the plasma and brain extracellular water when the molecular weight is < 200–400. Protein binding is again important since the drug takes on the configuration and size of the protein to which it is bound; therefore, the more important factor is the avidity with which a drug is bound to proteins and the ease with which it may become dissociated.

The concept of the blood-brain barrier has altered perceptibly over the last several years (5). First, the barrier clearly is not completely impermeable to substances, but rather is a selective and complex

DELIVERY OF A DRUG TO THE CENTERAL NERVOUS SYSTEM

G.I. TRANSIT (PH - CHEMICAL - ENZYMATIC)
TRAVERSE THE INTESTINAL WALL
SURVIVE HEPATIC CIRCULATION
RESIST DEGRADATION IN THE BLOOD STREAM (& OTHER ORGANS)
REMAIN UNIONIZED
EASILY DISSOCIATE FROM PLASMA PROTEINS
TRAVERSE CNS CAPILLARY EPENDYMA (TIGHT JUNCTIONS)
ENTER E.C.S. OF THE BRAIN (AQUEOUS·10-20%)
CROSS CELLULAR MEMBRANES
ENTER CYTOPLASMIC WATER
ARRIVE IN INTRACELLULAR WATER

FIGURE 1.—Sequence of aqueous and lipophilic conditions through which a drug must pass to be delivered to a brain tumor.

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[2]CCNU: NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-.

Methyl-CCNU: NSC-95441; CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-.

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phenomenon serving to regulate the internal milieu of the brain. Second, the blood-brain barrier does not specifically relate to brain tumor because of the changes within the capillary's neovascularization which takes place in and around the tumor. On the periphery of brain tumors, however, where normal architecture may be maintained, there may be islands of active tumor cells shielded from effective plasma concentrations of drug by the blood-brain barrier.

The physical and chemical characteristics of the three nitrosoureas are shown in figure 2. All of these are of roughly equivalent molecular weight. They are, however, approximately one log apart in their log P partition coefficients, thus indicating that CCNU is about ten times less water soluble than BCNU[3] and methyl-CCNU is about ten times less soluble than CCNU. Practically speaking, BCNU is marginally soluble in aqueous solution and CCNU and methyl-CCNU are not soluble without special preparation.

CCNU may be specifically labeled in each of three positions (6). In order to investigate its absorption, distribution, and concentration in cerebrospinal fluid (CSF) and brain tumor, the radiolabeled material has been orally administered to patients. Samples of blood, CSF, urine, and tissue were obtained at various times and analyzed by radiometric procedures previously described. There is a rapid absorption of the orally administered material with the peak plasma concentration found at 1 hour (fig 3). The early plasma half-life is approximately 10 hours, with a more prolonged plasma half-life of > 1 day. The protein binding is found to be 54% at the peak plasma concentration and 44% 4 hours later. The excretion of the ^{14}C label is rapid during the

[3]BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-.

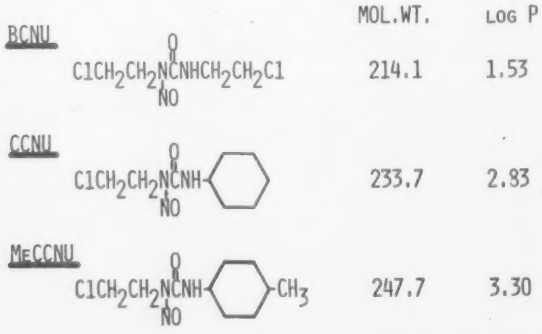


FIGURE 2.—Structure, molecular weight, and log P of the octanol/water partition coefficient of 3 nitrosoureas.

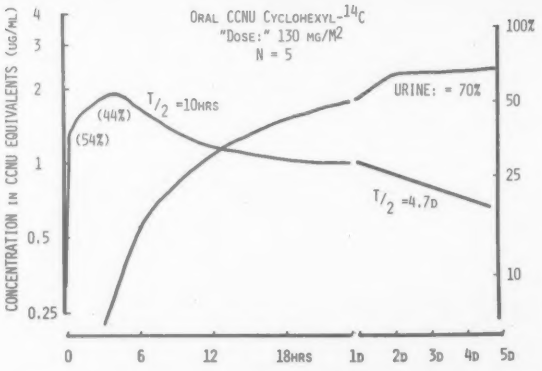


FIGURE 3.—Average plasma concentration of CCNU equivalents and urinary excretion of orally administered ^{14}C-cyclohexyl-CCNU in 5 patients.

first 24 hours, with the vast majority being excreted during that time and an average 5–7-day excretion total of 70%. Only traces of label are found within the stool and, therefore, it is reasonable to assume that the drug is relatively completely absorbed. Virtually none of the material found within the blood is considered to be intact compound, although 25% of the early plasma concentrations are ether soluble. They are now felt to represent the hydroxylated metabolic products of CCNU (7,8). Examination of the CSF of patients has indicated that the maximal CSF:plasma ratio is < 0.5, and there is an even more rapid loss of radiolabeled material within the CSF.

Studies have been performed that demonstrate the cumulation of radiolabeled CCNU in the murine glioma model tumor (9). Individual patients may also be studied during surgery in order to determine more precisely the characteristics of the entrance of these materials into tumor tissue. It must be recognized that these studies are inherently more difficult to perform and are subject to the influences of anesthesia, drug-drug interactions, blood replacements, and the effects which operative intervention may have upon pharmacodynamics. One example indicates prompt absorption of CCNU with a peak plasma concentration at 1 hour (fig 4). Several small samples of tumor (a highly malignant glioma) and adjacent muscle were taken during resection of the tumor in order to analyze the temporal events. At 1½ hours after administration of ^{14}C-chloroethyl-labeled CCNU, both the tumor and plasma have virtually equal concentrations of radiolabel, and at the 2- and 2½-hour intervals, increasing concentrations are found both in the glioma and in the adjacent muscle. It is clear that no barrier exists to the entrance of the labeled CCNU metabolites into

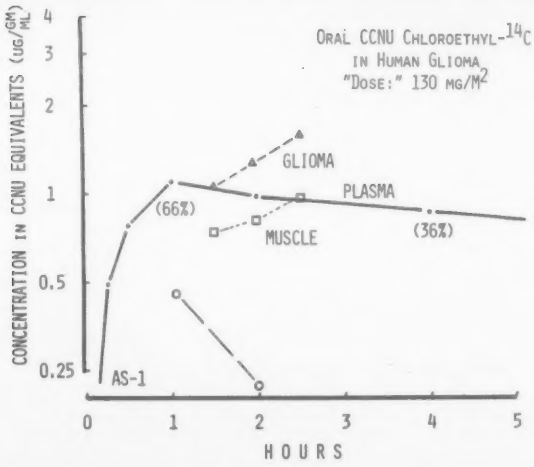


FIGURE 4.—Plasma, tumor, and muscle concentrations of orally administered ^{14}C-chloroethyl-CCNU to a patient.

malignant glioma. Plasma protein binding of approximately two thirds of the label does not interfere with accumulation within the tumor. Ether extraction of samples of this tumor failed to indicate any radioactivity, showing that neither the parent compound nor the hydroxylated metabolite was present.

A summary of the ^{14}C-CCNU distribution in glioblastoma at various times is shown in figure 5. CCNU labeled in both the chloroethyl and cyclohexyl positions shows a progressive increase in the tumor:plasma ratio during the time intervals examined. It is of interest and remains unexplained why the three patients receiving carbonyl-labeled CCNU have consistently low tumor:plasma ratios at all time intervals examined. It may be that the answers to these questions and more specific identification of metabolic products will be forthcoming with the development of high-pressure liquid chromatography and mass spectroscopy of the nitrosoureas.

The recent efforts of Hilton and Walker (10) and May et al (8) have indicated that the hydroxylation of the CCNU ring at various positions may well represent an intermediate product in the metabolism of this drug. Furthermore, techniques have been developed which allow the delivery of CCNU iv, thereby eliminating the gastrointestinal absorption and first-pass hepatic degradation (11). The plasma of four patients receiving therapeutic doses of CCNU iv was evaluated precisely at the end of the infusion for the currently identifiable metabolites of CCNU (fig 6). At the time of examination, approximately three quarters of the plasma concentration is the hydroxylated product and, therefore, hydroxylation takes place faster than the infusion rate of the drug. Approximately one third of the plasma appears as *cis*-4-hydroxy-CCNU while almost two thirds appear as *trans*-4-hydroxy-CCNU. Further elucidation of the products and rates of metabolism needs to be undertaken.

The pharmacologic studies described above and those which have been reported in the literature must rely primarily on the radioactive distribution of the nitrosoureas. Although this may have been the most precise tool available at the time, the data are relatively imprecise and do not provide the kinds of information needed in order to understand the pharmacodynamics of the nitrosoureas in relation to their mechanisms of action. Improved therapeutic regimens and treatment schedules and new nitrosourea derivatives cannot be rationally approached without a better understanding of the basic mechanisms of the nitrosoureas. With the newly developed techniques of separation, high-pressure liquid chromatography, and mass spec-

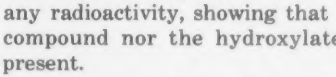
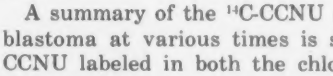
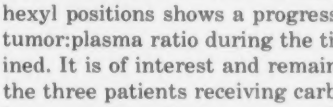
LABEL POSITION	TUMOR/PLASMA RATIO 30MIN	60MIN	120MIN
CHLOROETHYL 	0.60 ±.11	0.72 ±.06	1.21 ±.19
CYCLOHEXYL 	-	1.01 ±.16	1.50 ±.25
CARBONYL 	0.14 ±.02	0.14 ±.02	0.09 ±.02

FIGURE 5.—Tumor:plasma ratios of ^{14}C-chloroethyl-, cyclohexyl-, or carbonyl-labeled CCNU at various time intervals (mean ± SE).

IDENTIFIABLE METABOLITES OF CCNU
AT THE END OF A ONE HOUR INFUSION

PATIENT	TRANS 3-OH	CIS 4-OH	TRANS 4-OH
A	3%	36%	61%
B	5	37	58
C	4	31	65
D	6	28	66
MEAN	4.5%	33%	62.5%

FIGURE 6.—Distribution between the identifiable hydroxylated metabolites of iv administered CCNU in 4 patients.

troscopy, an understanding of some of the intermediate metabolites present is now within our reach.

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Therapy for Gastrointestinal Cancer With the Nitrosoureas Alone and in Drug Combination[1]

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Gastrointestinal cancer will affect almost 170,000 Americans during 1976 and cause 102,000 deaths (1). These figures represent more than one fourth of the total cancer incidence and mortality in this country. They greatly exceed those for cancer originating in any other organ system.

In spite of the magnitude of this problem, there has been little, if any, substantive progress in the treatment of gastrointestinal cancer over the past 30 years. Over 95% of all patients with primary carcinomas of the esophagus, pancreas, and liver still die of their disease, as do almost 90% of those with gastric carcinoma and 60% of those with colorectal carcinoma. Extensive public education programs and sophisticated diagnostic approaches have not succeeded in bringing patients to surgeons at an earlier stage of the disease. The results of surgical treatment, regardless of stage, have not shown any visible improvement for at least a quarter century. It is painfully evident that we must deal with gastrointestinal cancer as the patient presents it to us, and, in the main, this is not during a stage that is amenable to curative surgical treatment.

From the standpoint of nonsurgical treatment of gastrointestinal cancer, the radiation therapist has played only a small part. The immunologist waits hopefully in the wings, but it is still highly questionable that he will ever stand on center stage. The most immediate and tangible hope would seem to rest with chemotherapy.

For practical purposes, chemotherapy for gastrointestinal cancer originated with the development of 5-fluorouracil (5-FU) some 18 years ago. Certainly 5-FU has been amply demonstrated to have activity against gastrointestinal adenocarcinoma, but the frequency and degree of this activity have been too minor to justify anything more than academic interest. There is no evidence that 5-FU therapy contributes significantly to the longevity of the overall population of patients treated regardless of the stage of the disease at the time of treatment. Indeed, tenacious insistence by some that 5-FU should be regarded as routine treatment for gastrointestinal cancer has quite possibly impeded clinical progress in the management of this disease.

Among numerous alternative chemotherapeutic agents which have been studied, only mitomycin C and the nitrosoureas have appeared to show a frequency of objective response exceeding that which could be anticipated from investigator error in tumor measurement. Mitomycin C has been burdened by the problems of minimal response rates, durations of responses averaging only a very few weeks, and the rapid development of cumulative bone marrow toxicity. Its only possible role, not yet clearly established, has been in combination chemotherapy for gastric carcinoma. Our studies of the nitrosoureas in gastrointestinal cancer were initiated following the phase I studies of BCNU reported in 1965 by DeVita and associates (2). With the demonstration of some therapeutic activity for this agent, we then pursued the more recently developed analogs, CCNU and methyl-CCNU. To date we have studied > 300 patients treated with the nitrosoureas as single drugs, in addition to a still larger number who received the nitrosoureas in various combination regimens.

In evaluating our therapeutic results we employ the following criteria to declare an objective response: (*a*) There must be a reduction by at least 50% in the product of the longest perpendicular diameters of the most clearly measurable mass. (*b*) If malignant hepatomegaly is employed as an indicator, there must be a reduction by at least 30% in the sum of measurements below the xiphoid process and each costal margin. (*c*) If a liver scan is employed, it is required that at pretreatment there be a clearly measurable perfusion defect measuring at least 5 cm in greatest diameter. There must be a reduction by at least 50% in the product of the longest perpendicular diameters of this lesion. (*d*) No new areas of malignant disease may appear. (*e*) There may be no increase in other areas of known malignant disease, and this result must be observed at least 6 weeks after the start of therapy.

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Treatment of Gastrointestinal Cancer With the Nitrosoureas as Single Agents

In these single-agent studies (3) we employed BCNU at total doses ranging from 250 to 375 mg/m² given iv daily over periods ranging from 3 to 6 days; CCNU was given in a single oral dose of 130 mg/m²; and methyl-CCNU was given in a single oral dose ranging from 200 to 250 mg/m². Courses of therapy were repeated at intervals of between 6 and 8 weeks.

Toxic reactions to the three nitrosoureas were qualitatively and quantitatively similar. They consisted primarily of frequent nausea and vomiting during therapy, occasional mild and transient early leukopenia about 2 weeks after the start of therapy, thrombocytopenia with a nadir at 4 weeks, and delayed leukopenia reaching its nadir at 5–6 weeks. There was a definite tendency for hematologic toxic effects to compound with repeated treatment. This tendency seemed to be accentuated when shorter intervals between courses were employed and when severe marrow toxicity occurred with earlier courses.

Our overall objective response rates in gastrointestinal cancer are shown in table 1. The median duration of these responses was approximately 4 months. In our experience, methyl-CCNU was the most favorable nitrosourea for gastrointestinal adenocarcinoma. This apparent superiority for methyl-CCNU was also evident in three controlled studies in colorectal cancer in which we compared the drug against a common control, 5-FU (table 2). The only direct comparison of nitrosoureas in gastrointestinal cancer was conducted by the Acute Leukemia Group B who found a significant superiority of methyl-CCNU over CCNU in colorectal cancer.[3]

Our only experience with the nitrosoureas in squamous cell carcinomas of the gastrointestinal tract has been with CCNU (table 3). Considering the previous drug resistance of this group of neoplasms, this experience must be considered as quite favorable.

Although it would appear that the nitrosoureas do have some activity in gastrointestinal cancer, it is, nonetheless, apparent that their contribution as single-drug therapy is minimal and does not match the meager accomplishments of 5-FU. It would seem highly unlikely that within the foreseeable future any single-drug treatment will be of truly meaningful value to the patient with gastrointestinal cancer at any stage of the disease.

[3]Mortz G, Maurice P, and Holland JF (Acute Leukemia Group B). Personal communication, 1975.

TABLE 1.—Therapeutic results of gastrointestinal adenocarcinomas treated with the nitrosoureas as single agents

Drug	Primary carcinoma	No. of patients with objective response/No. treated: No prior chemotherapy	Prior chemotherapy	All patients
BCNU	Colorectal	4/22 (18%)	4/42 (9.5%)	8/64 (12.5%)
	Stomach	5/28 (18%)	1/5	6/33 (18.5%)
	Pancreas	0/22	0/9	0/31
	Biliary	2/4	—	2/4
	Hepatoma	0/3	—	0/3
	Carcinoid	0/1	0/1	0/2
	Total	11/80 (14%)	5/57 (9%)	16/137 (12%)
CCNU	Colorectal	3/29 (10%)	4/46 (9%)	7/25 (10%)
	Stomach	0/10	0/1	0/11
	Pancreas	1/2	1/2	2/4
	Biliary	—	0/4	0/4
	Hepatoma	0/1	—	0/1
	Small bowel	0/1	—	0/1
	Islet cell	—	0/1	0/1
	Total	4/43 (9%)	5/54 (9%)	9/97 (9%)
Methyl-CCNU	Colorectal	4/16 (25%)	3/24 (12.5%)	7/40 (17.5%)
	Stomach	0/9	2/6	2/15 (13%)
	Pancreas	1/6	1/9	2/15 (13%)
	Biliary	—	1/2	1/2
	Islet cell	0/1	2/4	2/5
	Total	5/32 (16%)	9/45 (20%)	14/77 (18%)

TABLE 2.—Comparative evaluation of the nitrosoureas vs 5-FU in advanced colorectal carcinoma

Drug	No. of patients with objective response/ No. treated
5-FU*	6/25 (24%)
BCNU*	3/24 (12.5%)
5-FU†	3/19 (16%)
CCNU†	2/28 (7%)
5-FU*	3/21 (14%)
Methyl-CCNU*	5/19 (26%)

*Randomized comparison.
†Fixed crossover comparison: CCNU to 5-FU.

TABLE 3.—CCNU therapy for advanced gastrointestinal squamous cell carcinoma

Primary carcinoma	No. of patients with objective response/ No. treated
Esophagus	3/19 (16%)
Anus	2/2
Pancreas	0/2
Gallbladder	0/1
Total	5/24 (21%)

Combination Chemotherapy for Gastrointestinal Cancer With 5-FU and the Nitrosoureas

In the initial evaluation of the nitrosoureas as constituents of drug combinations for gastrointestinal cancer (4), we found that 5-FU could be combined with BCNU at approximately 75% of the full dose of each without additive hematologic toxicity. In a randomized study (4) we were surprised to find that this combination was inferior to each single drug used for colorectal carcinoma (table 4). This paradoxical result has subsequently been confirmed by Lokich and associates (5). On the other hand, the combination achieved superior response rates in both pancreatic carcinoma (33%) and gastric carcinoma (41%) (6). In pancreatic carcinoma this increase in response rate was not associated with any increase in survival, but gastric carcinoma patients treated with the combination had a significant increase in long-term survival when compared to those treated with either a single drug or to clinically matched untreated patients.

Confirmation of significant therapeutic activity of 5-FU/nitrosourea combinations in upper gastrointestinal cancer has also been documented in subsequent studies by both the Eastern Cooperative Oncology Group (7) and the Southwest Oncology Group (8).

In view of the possibility raised in earlier studies that methyl-CCNU may be the most active nitrosourea in colorectal carcinoma, we pursued this possibility in a controlled evaluation involving 80 patients (9). In this study we compared treatment with 5-FU alone versus a combination of 5-FU, methyl-CCNU, and vincristine. Dosages for the combination were methyl-CCNU, 175 mg/m² on Day 1; vincristine, 1 mg/m² on Days 1 and 36; and 5-FU, 350 mg/m²/day on Days 1–5 and 400 mg/m²/day on Days 36–40. This cycle was repeated every 10 weeks with appropriate reduction in doses if toxicity was excessive during the initial cycle. Therapeutic results are shown in table 5. If we consider only those patients who were ambulatory and capable of working full- or part-time, the superiority in response rate of the combination regimen becomes even more impressive: 53% versus 23% for 5-FU alone.

In a similar controlled study of 91 patients with colorectal carcinoma, Falkson obtained a 37% response with the 5-FU, methyl-CCNU, and vincristine combination compared to 22% with 5-FU alone.[1] The role of vincristine in this combination has yet to be established. Vincristine alone has not been demonstrated to have activity in colorectal carcinoma and the Southwest Oncology Group has demonstrated a significant advantage of 5-FU plus methyl-CCNU without vincristine over 5-FU alone (8).

[1]Falkson G. Personal communication.

TABLE 4.—Controlled evaluation of 5-FU, BCNU, or 5-FU + BCNU in gastrointestinal carcinoma

Primary carcinoma	No. of patients with objective response/No. treated		
	BCNU	5-FU	5-FU + BCNU
Colorectal	3/17 (18%)	5/20 (25%)	1/20 (5%)
Pancreas	0/21	5/31 (16%)	10/30 (33%)
Stomach	4/23 (17%)	8/28 (29%)	14/34 (41%)

TABLE 5.—Combined 5-FU, methyl-CCNU, and vincristine therapy for advanced colorectal carcinoma

Regimen	No. of patients with objective response/No. treated
5-FU	8/41 (19.5%)
5-FU + methyl-CCNU + vincristine	17/39 (43.5%) $P < 0.05$

DISCUSSION

Like 5-FU, the nitrosoureas have shown definite but clinically insignificant antineoplastic activity when used alone in the treatment of advanced gastrointestinal carcinoma. Combinations of either BCNU or methyl-CCNU with 5-FU have shown repeatedly confirmed superiority over single-drug therapy in upper gastrointestinal carcinomas as has the combination of methyl-CCNU with 5-FU in colorectal carcinoma. In advanced colorectal carcinoma, however, this superiority has been seen only in tumor regressions, not in patient survival. Only in gastric carcinoma does there seem to be some contribution to patient longevity, and this is relatively minor. While perhaps a small step forward, combined 5-FU and nitrosourea therapy does not merit classification as standard treatment for patients with advanced gastrointestinal cancer. Patients with this stage of disease should continue to be approached as challenges for clinical research.

It is possible that combined 5-FU and nitrosourea therapy may offer hope as a surgical adjuvant. The increases in therapeutic activity demonstrated by these combinations may be capable of increasing cure rates in the patient with minimal residual disease where treatment with 5-FU alone and other single drugs has failed. Such surgical-adjuvant studies are currently being carried out by several cooperative groups. It must be emphasized, however, that the effectiveness of 5-FU/nitrosourea surgical-adjuvant therapy is unproven at this time and the possible long-term hazards of this treatment remain unknown.

LIST OF COMPOUNDS

BCNU: NSC-409962; CAS reg. No. 154–93–8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-

CCNU: NSC-79037; CAS reg. No. 13010–47–4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-

5-Fluorouracil: NSC-19893; CAS reg. No. 51–21–8

Methyl-CCNU: NSC-95441; CAS reg. No. 33073–59–5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-

Mitomycin C: NSC-26980; CAS reg. No. 50–07–7

Vincristine: NSC-67574; CAS reg. No. 2068–78–2; leurocristine, sulfate (1:1)

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Randomized Prospective Trial Comparing 5-Fluorouracil (NSC-19893) to 5-Fluorouracil and Methyl-CCNU (NSC-95441) in Advanced Gastrointestinal Cancer[1,2]

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SUMMARY

The Southwest Oncology Group has confirmed the superiority of 5-fluorouracil (5-FU) and methyl-CCNU versus 5-FU weekly as treatment for patients with advanced gastrointestinal cancers. The drug combination produced a PR rate of 29.3% compared to the single-drug arm rate of 10.6%; however, the two-drug arm produced much more toxicity. Despite the improved response rate, no improvement in survival was observed.

[Cancer Treat Rep 60:733–737, 1976]

Since the introduction of 5-fluorouracil (5-FU) in 1957, systemic chemotherapy for advanced gastrointestinal (GI) adenocarcinomas has been extensively attempted in many institutions (1–5). However, despite the clinical availability of 5-FU for almost 20 years, controversy still exists regarding the most beneficial dosage, schedule, and route of administration. Several regimens have been devised to use 5-FU without producing a significant change in the clinical worthwhile tumor remission rates (6–9). Initially, Curreri et al reported an iv schedule for 5 days followed by every-other-day administration until toxicity occurred. Jacobs et al (10) used a weekly iv schedule, and further study (11,12) revealed that weekly iv 5-FU induced essentially the same tumor response as the initial schedule. This weekly schedule, because of its convenience and relative lack of toxicity, is now the schedule most frequently used by the practicing physician. However, in any large published series the response rate rarely exceeds 20% regardless of dose, schedule, or route of administration employed. Recently, the rationally synthesized nitrosourea, methyl-CCNU, has been shown to have modest activity in colorectal carcinomas (13,14). Wasserman et al (15) reviewed the overall experience with methyl-CCNU and found an 11% response rate. Moertel et al (16) have reported that 5-FU given daily × 5 with the addition of methyl-CCNU and vincristine has had a superior response rate compared to 5-FU given alone by the same schedule. The Southwest Oncology Group, because of the popularity of the weekly 5-FU, designed a stratified, randomized clinical trial of weekly 5-FU with a single intermittent dose of methyl-CCNU. The initial results from this study have recently been reported (17).

[1]Supported in part by Public Health Service grants CA-16943, CA-12014, CA-04915, CA-14028, CA-13612, CA-16385, CA-03392, CA-12644, CA-13643, CA-16975, CA-10187, CA-03096, CA-12213, CA-04920, CA-13238, and CA-04919 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[2]5-Fluorouracil: CAS reg. No. 51–21–8.

Methyl-CCNU: CAS reg. No. 33073–59–5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-.

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[5]The following institutions participated in this study; the number of patients is in parentheses: Henry Ford Hospital (44); Wayne State University (32); University of Arizona (22); Borgess Medical Center (20); University of Mississippi (20); Baylor College of Medicine (18); University of Kansas (18); University of Virginia (18); University of Oklahoma (17); Scott and White Clinic (15); Wilford Hall USAF (15); University of Texas at Galveston (13); Brooke Army Medical Center (11); University of New Mexico (11); Ohio State University (7); University of Arkansas (6); Northwestern University (4); Kessler USAF (3); University of Utah (3); and Cleveland Clinic (1).

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MATERIALS AND METHODS

From March 1973 to October 1974, 299 patients with disseminated GI cancers were entered in this protocol. Patients must have had histologic confirmation of an adenocarcinoma originating from the GI tract with firm evidence of a metastatic lesion that was measurable. Patients were not eligible for the study if they had received any prior chemotherapy or if they had received extensive radiotherapy in the immediate past. Patients were also excluded if their expected survival was < 90 days or if their tumors produced symptoms that required special care and assistance (Karnofsky performance status, < 50%). After entry in this study the patients with primary tumor in the colon or rectum were also stratified into two groups; patients with metastatic disease predominantly in the liver were randomized separately from the remaining group. Other groups of patients included those with gastric, pancreas, or other GI lesions. Within each group the patients were randomly allocated on a three to one basis for patients receiving the combination therapy (5-FU plus methyl-CCNU) versus the single-drug therapy (5-FU). This enabled us to have a more precise estimate of the response rate of the combination therapy than a one to one randomization would have allowed and also allowed a comparison of the 5-FU-alone patients with those from other studies. Pretreatment evaluation included a complete blood cell count with differential, platelet count, urinalysis, liver and renal function studies, and enzymatic chemistries that would be helpful in following the progress of the disease. During the study the analysis included weekly white blood cell (wbc) and platelet counts, tumor measurements at 2–3-week intervals, and serial blood chemistries at 6-week intervals. The effects of tumor therapy were evaluated using standard criteria, ie, only those patients who were accepted into the objective remission group had measurable tumors diminish by at least 50% of the sums of the tumor's greatest diameters without any other tumors or symptoms increasing. Patients with liver metastasis as the sole site of measurable tumor were evaluated by the method of Moertel. The distance between the lower liver edge and the rib edge (or xiphoid process) was measured at three points, each 8 cm from the other. The diminution of the sums of their measurements by at least 30% was required for an objective remission. Disappearance of all tumor signs was required for CR. Therapy for the combination group consisted of methyl-CCNU (175 mg/m^2 orally every 6 weeks) and 5-FU (400 mg/m^2 iv bolus weekly or until the wbc count fell to < 2000 cells/mm^3). The patients on the single-drug arm received 5-FU (400 mg/m^2/week iv) and doses were increased every 6 weeks by 100 mg/m^2 if no toxic effects were observed. Comparison was made between the two treatment arms regarding age, sex, and, for patients with colorectal lesions, anatomic site of the primary. Twenty-one of 40 patients (52%) receiving 5-FU alone were men compatients (52%) receiving 5-FU alone were men compared to 95 of 151 (63%) receiving the combination therapy. No statistical difference existed between sex distribution among the treatment arms ($P >$ 0.3). The average age for the patients receiving 5-FU alone was 59.3 years compared with 60.1 years for patients receiving the combination treatment. For patients receiving 5-FU alone the most frequent colorectal sites were sigmoid (14 of 40 patients) and rectum (12 of 40). For patients receiving the combination therapy the most common sites were the same: sigmoid (45 of 144 patients) and rectum (36 of 144). The patients from the two treatment arms were quite comparable with regard to the site of the primary colonic lesions ($P > 0.5$). The pattern of metastasis in the 194 patients with colorectal carcinomas showed that the liver was the most common site of metastasis (58.8%) followed by the lungs (25.3%), superficial lymph nodes (20.6%), and abdominal tumors (17%). The less frequent sites, ranging from 8% to 1.6%, included in decreasing order of frequency: deep soft tissue or lymph nodes, bones, central nervous system, pelvic area, organs, skin, and other.

RESULTS

We were able to make an evaluation as to response from this therapy in 279 of the 299 patients entered in this study. The eligibility criteria were not met in five patients. Of the 294 eligible patients, 15 (5%) were not evaluable for response because of early death (death occurring within 6 weeks from the start of therapy from causes other than therapy) or loss to followup. A summary of the responses can be seen in tables 1 and 2. In combining all groups of patients in the study the patients receiving 5-FU alone had a response rate of 10.6% (seven of 66 patients) compared to a response rate of 29.3% (62 of 212) for patients receiving the combination therapy. All responses were PRs. The difference between the two treatment arms was highly significant ($P < 0.01$).

As expected, 5-FU, when used in this manner alone, produced very little hematologic toxicity. In the 5-FU-alone group, 32% of the patients had GI toxicity which was usually mild. Nearly all patients receiving methyl-CCNU experienced nausea and vomiting. The two-drug combination produced significant myelosuppression. The mean nadir of leuko-

TABLE 1.—Response in colorectal carcinoma

Group*	Metastasis	Treatment	No. of patients— Eligible for treatment	No. of patients— Evaluable	No. of patients with— Increasing disease	No. of patients with— No change	No. of patients with— PR	% response Eligible patients	% response Evaluable patients	95% confidence limits
1	Liver	5-FU	11	11	7	4	0	0	0	
2	Liver	5-FU + methyl-CCNU	43	41	21	7	13	30.2	31.7	17.4–46.0
3	Other	5-FU	33	31	19	6	4	12.1	12.9	1.1–24.7
4	Other	5-FU + methyl-CCNU	100	111	58	17	35	29.2	31.5	23.1–40.6

*Groups 1 + 3 = 4 of 42 patients = 9.5%
Groups 2 + 4 = 48 of 152 patients = 31.6%
$P = 0.009$.

TABLE 2.—Response in upper GI cancers

Group*	Cancer	Treatment	No. of patients— Eligible for treatment	No. of patients— Evaluable	No. of patients with— Increasing disease	No. of patients with— No change	No. of patients with— PR	% response Eligible patients	% response Evaluable patients	95% confidence limits
5	Gastric	5-FU	10	9	7	0	2	20.0	22.2	0–49.4
6	Gastric	5-FU + methyl-CCNU	29	29	20	3	6	20.7	20.7	5.9–35.5
7	Pancreas	5-FU	6	6	4	1	1	16.7	16.7	0–46.5
8	Pancreas	5-FU + methyl-CCNU	16	15	10	2	3	18.6	20.0	0–40.3
9	Other	5-FU	9	9	6	3	0	0	0	—
10	Other†	5-FU + methyl-CCNU	18	17	10	2	5	27.8	29.4	7.8–51.1

*Groups 5, 7, and 9 = 3 of 24 patients = 12.5%
Groups 6, 8, and 10 = 14 of 61 patients = 23.0%
$P = 0.22$.
Groups 1, 3, 5, 7, and 9 = 7 of 66 patients = 10.6%
Groups 2, 4, 5, 8, and 10 = 62 of 212 patients = 29.3%
$P = 0.006$.
†Other sites included carcinomas of the liver, gallbladder, bile duct, ampulla of Vater, and small intestine. Responses were observed in carcinoma of the liver, bile duct, and small intestine.

penia and thrombocytopenia for the two-drug group was 3.2×10^3 cells/mm^3 (wbc) and 103×10^3 cells/mm^3 (platelet). The median number of days to nadir was 25 days for leukopenia and 22 days for thrombocytopenia. The two-drug therapy was cumulatively myelosuppressive. This necessitated a protocol amendment reducing the dose of methyl-CCNU to 125 mg/m^2 at course No. 3. The protocol required the doses of 5-FU to be omitted if the wbc count was < 2000 cells/mm^3. Most patients received > 1600 mg/m^2 of 5-FU with each course of nitrosourea. The total dose of 5-FU in our combination exceeded the total dose of 5-FU in the combination of Moertel.

The median days of remission was 108 days for the single-drug therapy and 137 days for the two-drug arm. Despite the significant improvement in the response rate with the two-drug combination versus the single drug, no statistically significant difference existed in survival between the two treatment arms. Figures 1–4 demonstrate the survival curves for the groups of patients. The median survival time for the combination group was 26 weeks compared with 21 weeks for patients receiving 5-FU alone ($P = 0.38$). For upper GI lesions the median survival for patients receiving the combination therapy as well as those receiving the single-drug arm was 18 weeks. In colorectal lesions the median survival was 30 weeks for patients receiving the combination treatment compared with 19 weeks for patients receiving 5-FU alone ($P = 0.81$).

DISCUSSION

We have been able to confirm the initial report of Moertel of the superior response rate attained with the combination of 5-FU and methyl-CCNU in com-

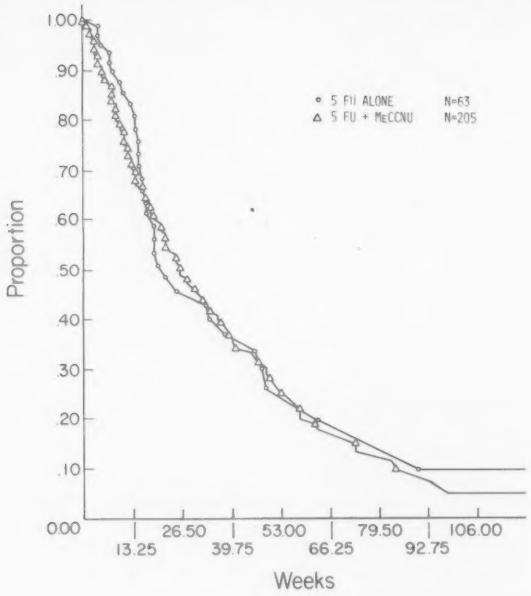

FIGURE 1.—Survival curve for all patients receiving 5-FU alone versus 5-FU plus methyl-CCNU.

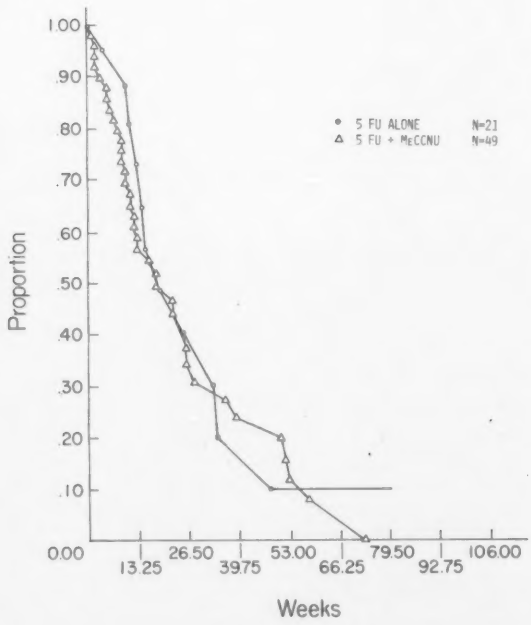

FIGURE 2.—Survival curve for patients with upper GI cancers using 5-FU alone versus 5-FU plus methyl-CCNU.

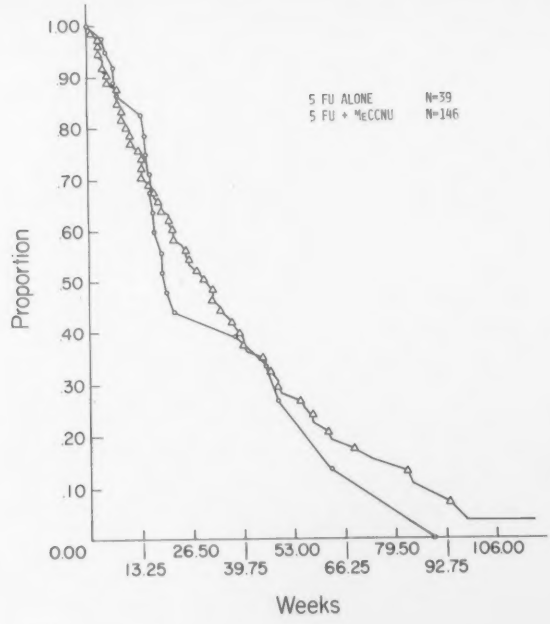

FIGURE 3.—Survival curve for patients with colorectal cancer using 5-FU alone versus 5-FU plus methyl-CCNU.

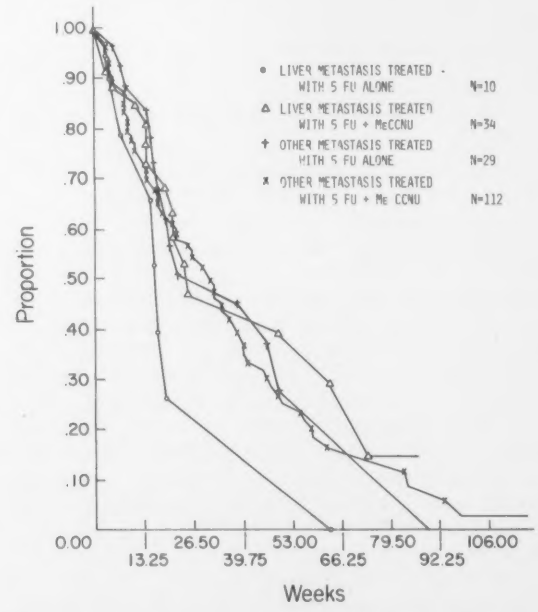

FIGURE 4.—Survival curve for patients with colorectal cancer with metastasis using 5-FU alone versus 5-FU plus methyl-CCNU.

parison to a weekly 5-FU schedule in GI cancers. It was, however, disappointing not to see an increase in survival between the two treatment arms although in each survival curve the tendency was for the combination arm to do better than the single-drug arm. This combination may represent an improvement in the treatment of advanced GI cancers.

ADDENDUM

Recent analysis by Moertel (personal communication) also failed to reveal an increase in survival of patients treated with 5-FU and methyl-CCNU compared to patients treated with 5-FU alone.

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Clinical Trials With BCNU (NSC-409962) in Malignant Lymphomas by the Eastern Cooperative Oncology Group[1]

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There has been considerable interest in the nitrosoureas by members of the Eastern Cooperative Oncology Group (ECOG) for > 10 years. Preliminary trials were conducted in the early 1960s utilizing three dosage schedules in 144 patients with advanced malignancies (1). One patient with mycosis fungoides achieved a complete remission (CR), two of four patients with Hodgkin's disease had partial responses (PR), and no responses were noted in three patients with lymphosarcoma. The overall response rate was 21% with several dramatic remissions of relatively short duration (6–8 weeks). The doses employed ranged from 250 to 300 mg/m² × 1 day.

A combined ECOG-Acute Leukemia Group B phase II study was carried out in 1968 in patients with malignant lymphomas (2). Several dosage regimens were employed (168 or 250 mg/m² iv every 6 weeks; 60 or 90 mg/m² iv weekly × 6). Of 44 randomized patients, 19 (43%) responses were recorded with five CRs. The breakdown of responses included 16 of 33 (48%) in Hodgkin's disease, two of seven in lymphosarcoma, and one of four in reticulum cell sarcoma. No evidence of cross resistance to prior alkylating agents (specifically to cyclophosphamide and to mechlorethamine) was noted.

With these studies as a background, three major phase III studies were initiated in 1972 by the ECOG: EST 1472, EST 2472, and EST 3472. All three programs utilized BCNU as part of a combination chemotherapy arm.

EST 1472, a study restricted to lymphocytic lymphomas (both nodular and diffuse) but excluding histiocytic lymphomas and Hodgkin's disease, was designed to answer the following questions: (*a*) Is BCNU, given in combination with prednisone and referred to as BP, as effective an inducer as cyclophosphamide and prednisone (CP) in the treatment of lymphocytic lymphomas? (*b*) After moderate induction, will intensive cyclic chemotherapy with BCNU, cyclophosphamide, vincristine, and prednisone (BCVP) prove superior to 6 months of intermittent chlorambucil treatment in improving the quality of responses (ie, changing PRs to CRs) and prolonging the disease-free interval (3)? The treatment schedule consisted of prednisone (100 mg/m²/day for 5 days orally) with either BCNU (120 mg/m² iv) or cyclophosphamide (1000 mg/m² iv) every 4 weeks. After three cycles of treatment (ie, 3 months) patients with CR and PR were randomized into the maintenance phase with either chlorambucil (18 mg/m² orally every 2 weeks) or BCVP (BCNU, 60 mg/m²; cyclophosphamide, 600 mg/m²; vincristine, 1.4 mg/m²; and prednisone, 100 mg/m²/day for 5 days).

Three hundred and fifty-nine patients were evaluated (table 1). The CR rates were 18% and 23% for BP and CP, respectively (no significant difference). The overall response rates for initial induction were 56% and 67% for BP and CP, respectively. The CR rates were 9% for those patients who had had prior chemotherapy and 24% for patients who had not received chemotherapy, a significant difference. Some hematologic toxic effects were experienced by almost one half of the patients but these effects rarely were serious. No meaningful differences were observed between the treatment arms.

Overall, 77% of the patients have survived for at least 1 year with no difference noted between the induction arms. By histology, patients with a nodular architecture fared better than those with a diffuse pattern. Patients who failed to respond to one induction treatment were crossed over to the other induction arm; similar but slightly lower response rates were noted (45% for BP and 49% for CP), demonstrating the lack of cross resistance. These results improved the overall response rate prior to maintenance to 81%, of which 28% were CRs.

In the maintenance program, three times as many patients relapsed who were receiving chlor-

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TABLE 1.—Objective tumor responses in 359 evaluable patients (EST 1472)

	Treatment (No. of patients)					
	Initial		Crossover		Phase 2	
Type of response	BP	CP	BP	CP	BCVP	Chlorambucil
Complete	32 (18%)	43 (23%)	10 (14%)	14 (19%)	41 (32%)	19 (15%)
Partial	66	80	23	23	0	0
No change	25	19	17	17	31	29
Progression	51	40	20	19	54	74
Unknown	1	2	4	2	1	1
Total	175	184	74	75	127	123

ambucil compared to those receiving BCVP. An additional 24% of all patients entered CR during phase 2 of the study: 41 patients (32%) receiving BCVP and 19 patients (15%) receiving chlorambucil. Considering all combinations of induction plus maintenance treatment, an estimated 44% of all patients had a CR.

This study demonstrates that BCNU is as good as cyclophosphamide when used as an inducing agent for lymphocytic lymphomas. In addition, there was no cross resistance in those patients who failed inductions with either regimen. The four-drug combination of BCVP appears superior to chlorambucil in the second phase or consolidation phase of the study by the higher percentage of CRs and fewer relapses on the intensive arm during treatment. However, after all treatment ceased, relapses occurred at the same rate in both treatment arms.

EST 3472 was an ECOG phase III study initiated to evaluate moderate versus intensive chemotherapy in histiocytic lymphomas (4). The objectives were to compare the induction potential of the two-drug combination of CP, the three-drug combination of CVP (cyclophosphamide, vincristine, and prednisone), and the four-drug combination of BCVP; the durations of CRs were also compared. The treatment schedule is outlined in table 2. The drugs were administered every 3 weeks in contrast to the every 4-week schedule in EST 1472, and the dosage of cyclophosphamide was 40% higher in this four-drug BCNU-containing combination.

TABLE 2.—Evaluation of moderate vs intensive chemotherapy in histiocytic lymphoma (reticulum cell) sarcoma (EST 3472)

Treatment	Dose	Schedule
A. CP		Every 21 days
Cyclophosphamide	1 g/m²/day iv	
Prednisone	100 mg/m²/day × 5 orally	
B. CVP		Every 21 days
Cyclophosphamide	1 g/m²/day iv	
Vincristine	1 mg/m²/day iv	
Prednisone	100 mg/m²/day × 5 orally	
C. BCVP		Every 21 days
BCNU	60 mg/m²/day iv	
Cyclophosphamide	1 g/m²/day iv	
Vincristine	1 mg/m²/day iv	
Prednisone	100 mg/m²/day × 5 orally	

As of November 1975, 186 cases out of 228 accruals have been analyzed. There has been a greater incidence of hematologic toxicity with the four-drug combination, 37% of patients having severe toxic effects against 19% of patients in the other treatment arms. All four deaths were observed in the BCVP program.

A very high percentage (43%) of patients failed to complete 3 months of treatment; most patients progressed to death from disease. Fifty-four patients (29%) entered CR, with an overall response rate of 48% (table 3). The response rates were higher with both the three- and four-drug programs, but only in the number of CRs (34% versus 20%). An additional five patients (10%) who had failed inductions with either CP or CVP entered CR after BCVP treatment.

Patients with a nodular histology had a higher CR rate (45%) than those with a diffuse pattern (28%). Over 80% of the responses were achieved within the first 3 months of study, but one half of these patients had relapsed within the first year of treatment. Sixty-six percent of patients with a nodular histology were alive at 1 year compared to 38% of patients with a diffuse histology. Approximately 75% of all analyzed cases have been reviewed by a member of the Pathology Panel for Combined Clinical Trials. The estimated median survival time for the entire group is < 1 year.

This study demonstrates a lower response rate, a rapid recurrence, and a short survival time for histiocytic lymphomas. The addition of vincristine to CP (CVP) produces significant improvement in CRs

TABLE 3.—Treatment results of EST 3472

Type of response	No. of patients receiving drug regimen—			
	CP	CVP	BCVP	Totals
Complete	13 (20%)	20 (34%)	21 (33%)	54 (29%)
Partial	10 (16%)	14 (23%)	11 (18%)	35 (19%)
No change	5	4	10	19
Progression	33	21	18	72
Unknown	3	0	3	6
Total	64	59	63	186

and survival, without increased toxicity. The addition of BCNU (BCVP) contributes increased toxicity without increased remission. A nodular pattern is predictive of improved response rates and survival.

The EST 2472 study was initiated to compare standard MOPP (mechlorethamine, vincristine [Oncovin], procarbazine, and prednisone) combination chemotherapy with a five-drug combination of BCNU, cyclophosphamide, vinblastine, procarbazine, and prednisone (BCVPP) for the frequency of CR, the crossover-induction potential in nonresponders, and the value of BCVPP versus bacillus Calmette-Guérin (BCG) or no therapy once CR was obtained in patients with Hodgkin's disease (fig 1).

The dosage and schedule of the MOPP program were identical to the original regimen described by DeVita et al (1) (table 4). The BCVPP combination was identical to one developed by the Southeastern Cancer Study Group: SEG protocol 340 (table 5). The major differences from the MOPP schedule were the substitution of cyclophosphamide (600 mg/m²) for mechlorethamine, the substitution of vinblastine (5 mg/m²) for vincristine, and the addition of BCNU (100 mg/m²). Unlike the MOPP program, iv medication was administered only once per month.

This study (EST 2472) was activated in April 1972. As of October 1975, 230 patients were evaluable for induction treatment: 31 for the crossover treatment

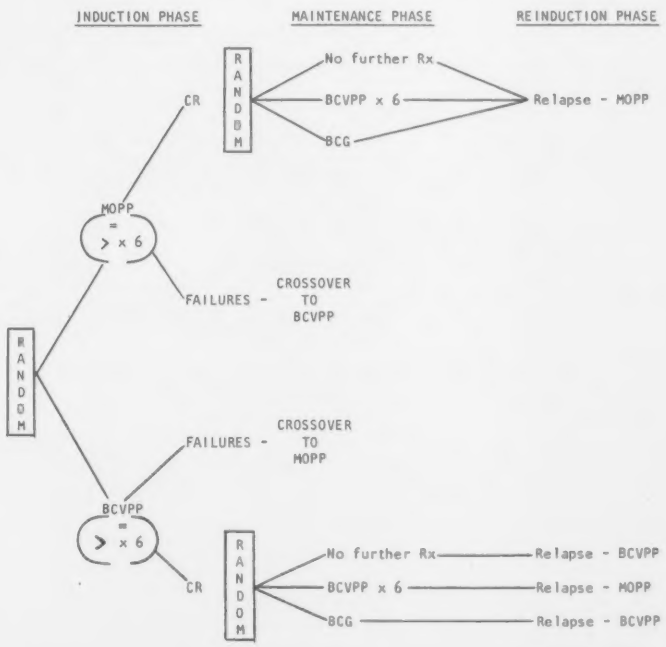

FIGURE 1.—Evaluation of combination chemotherapy and immunotherapy in patients with Hodgkin's disease (EST 2472).

step and 91 for the maintenance phase. There were three major types of toxicity noted. There was no significant difference in the degree of hematologic side effects (table 6) with moderate-to-severe pancytopenia occurring in about 60% of patients with both regimens. However, there were far fewer instances of gastrointestinal disturbances (nausea, vomiting, or diarrhea) with BCVPP and a very low incidence of central nervous system toxicities (primarily the result of the substitution of vinblastine for vincristine) with the BCVPP regimen.

CR was obtained in 68% of patients receiving the induction treatment and 23% of those receiving the crossover treatment. Including the crossover data 72% of all patients had CRs. The overall response rate was 87% (table 7). CR was recorded within 6 months for 70% of patients. There was no significant difference between the induction programs in achieving either CR or PR. Neither the extent of prior treatment nor the histologic cell type influenced response rate. Among the evaluable patients who received maintenance therapy, 30% have relapsed. There are no important differences in the relapse rates between the inducing regimens, and no conclusions can be drawn at this time as to the value of either BCG or BCVPP in maintaining remissions.

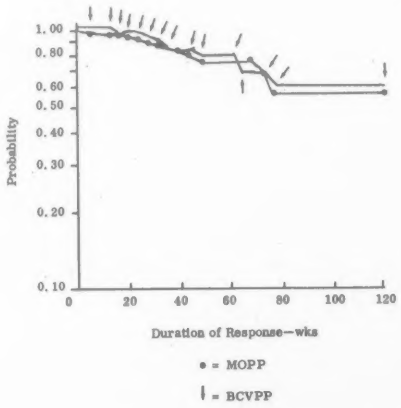

FIGURE 2.—Duration of response in Hodgkin's disease patients who entered maintenance (EST 2472).

TABLE 4.—Hodgkin's disease chemotherapy—MOPP regimen*

Drug	Dosage	Day— 1	8	14	28
Mechlorethamine	6 mg/m² iv	×	×		
Vincristine	1.4 mg/m² iv (max, 2 mg)	×	×		
Procarbazine†	100 mg/m² orally (14 days)	× × × × × × ×	× × × × × × ×		
Prednisone	40 mg/m² orally (14 days, cycles 1 and 4)	× × × × × × ×	× × × × × × ×		

*Repeat cycle every 28 days.
†50 mg on Day 1, 100 mg/m²/day thereafter.

TABLE 5.—Hodgkin's disease chemotherapy—BCVPP regimen*

Drug	Dosage	Day— 1	10	28
BCNU	100 mg/m² iv	×		
Cyclophosphamide	600 mg/m² iv	×		
Vinblastine	5 mg/m² iv	×		
Procarbazine†	100 mg/m² orally (10 days)	× × × × × × × × × ×		
Prednisone	60 mg/m² orally (10 days, all cycles)	× × × × × × × × × ×		

*Repeat cycle every 28 days.
†50 mg on Day 1, 100 mg/m²/day thereafter.

TABLE 6.—Toxicity of EST 2472

Toxicity type	Degree*	% of patients with toxicity who received— MOPP	BCVPP
Hematologic	0–1	37	39
	2–4	63	61
Gastrointestinal	0–1	51	71
	2–4	49	29
Central nervous system	0–1	74	96
	2–4	26	4

*0 = none; 1 = mild; 2 = moderate; 3 = severe; 4 = life-threatening.

TABLE 7.—Objective response—induction and crossover (EST 2472)

Type of response	Treatment regimen (No. of patients) Induction MOPP	BCVPP	%	Crossover MOPP	BCVPP	%
Complete	79	78	68	5	2	23
Partial	13	16	13	1	5	19
No change	5	6	5	4	2	19
Progression	11	9	9	3	9	39
Not evaluable	7	6	5	0	0	0
Totals	115	115	100	13	18	100

Because of the large number of continuing responses and insufficient followup time, the median duration of CR cannot be reliably estimated at this time. However, preliminary estimates suggest that there is approximately a 50% chance of the remissions lasting for at least 2 years (fig 2).

In this randomized prospective ECOG phase III trial, BCVPP had equal induction capability when compared to the standard National Cancer Institute MOPP program. Overall toxicity appears to be less, with the potential advantage of a once-monthly treatment program. The median duration of CR is at least 24 months.

In October 1974, two new ECOG non-Hodgkin's lymphoma studies were activated. EST 2474 (fig 3) is examining the value of moderate versus intensive induction therapy for favorable histologic subtypes (nodular forms and diffuse well-differentiated lymphocytic lymphoma). EST 3474 (fig 4) is comparing four programs of intensive chemotherapy for unfavorable histologic subtypes (diffuse lymphocytic, mixed, and histiocytic forms). BCVP is common to both studies in the induction phase and also in the maintenance arm, and is compared to no treatment after eight cycles of induction treatment. The dosage schedules for the favorable lymphoma study are outlined in table 8. Note that the BCVP regimen is identical to the one used in EST 3472 with the

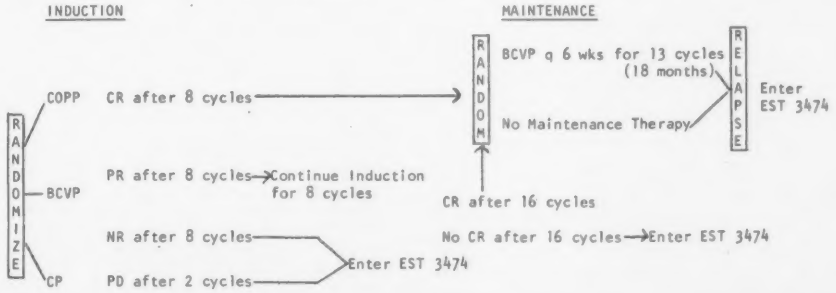

FIGURE 3.—Chemotherapy of favorable lymphomas (nodular lymphocytic poorly differentiated, nodular mixed, nodular histiocytic, nodular lymphocytic well differentiated, and diffuse lymphocytic well differentiated) (EST 2474).

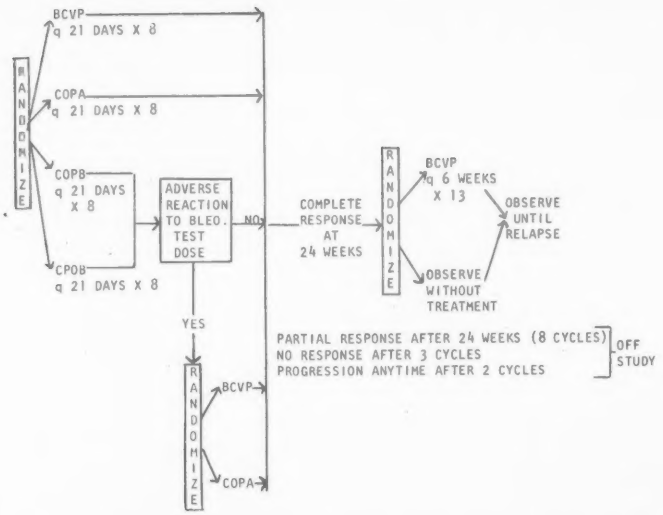

FIGURE 4.—Evaluation of 4 intensive chemotherapy regimens for the treatment of unfavorable histologic subtypes of malignant lymphoma (EST 3474).

TABLE 8.—Chemotherapy of favorable lymphomas (nodular lymphocytic poorly differentiated, nodular mixed, nodular histiocytic, nodular lymphocytic well differentiated, and diffuse lymphocytic well differentiated)

Drug regimen	Dose and schedule
COPP (repeat cycles every 28 days × 8)	
Cyclophosphamide	600 mg/m² iv, Days 1 and 8
Vincristine*	1.2 mg/m² iv, Days 1 and 8
Procarbazine	100 mg/m²/day orally × 14 days
Prednisone	100 mg/m²/day orally × 14 days
BCVP (repeat cycles every 21 days × 8)	
Cyclophosphamide	1000 mg/m² iv, Day 1
BCNU	60 mg/m² iv, Day 1
Vincristine*	1.2 mg/m² iv, Day 1
Prednisone	100 mg/m² orally, Days 1–5
CP (repeat cycles every 28 days × 8)	
Cyclophosphamide	600 mg/m² iv, Days 1 and 8
Prednisone	100 mg/m²/day orally × 14 days

*Individual doses not to exceed 2.0 mg.

treatment given every 21 days rather than every 28 days. The COPP (cyclophosphamide, vincristine, [Oncovin], procarbazine, and prednisone) program is modeled after the one by Schein and co-workers (5).

In EST 3474, we have an identical common BCVP arm and three additional programs: COPA (cyclophosphamide, vincristine [Oncovin], prednisone, and adriamycin) (a Southwest Oncology Group program also referred to as "CHOP") and two arms using bleomycin and CVP, one regimen with the bleomycin and vincristine administered 2 weeks after cyclophosphamide and prednisone (table 9). It is premature to consider the treatment results of either study at this time.

TABLE 9.—Evaluation of 4 intensive chemotherapy regimens for the treatment of unfavorable histologic subtypes of malignant lymphoma

Drug regimen	Dose and schedule
BCVP (repeat cycle every 21 days × 8)	
BCNU	60 mg/m² iv, Day 1
Cyclophosphamide	1000 mg/m² iv, Day 1
Vincristine*	1.2 mg/m² iv, Day 1
Prednisone†	100 mg/m²/day orally, Days 1–5
COPA (repeat cycle every 21 days × 8)	
Cyclophosphamide	600 mg/m² iv, Day 1
Vincristine*	1.2 mg/m² iv, Day 1
Prednisone†	100 mg/m² orally, Days 1–5
Adriamycin	50 mg/m² iv, Day 1
COPB (repeat cycle every 21 days × 8)	
Cyclophosphamide	1000 mg/m² iv, Day 1
Vincristine*	1.2 mg/m² iv, Day 1
Prednisone†	100 mg/m²/day orally, Days 1–5
Bleomycin‡	10 mg/m² im, Day 1
CPOB (repeat cycle every 21 days × 8)	
Cyclophosphamide	1000 mg/m² iv, Day 1
Prednisone†	100 mg/m²/day orally, Days 1–5
Vincristine*	1.2 mg/m² iv, Day 15
Bleomycin‡	10 mg/m² im, Day 15

*Individual doses not to exceed 2.0 mg.
†Single daily dose.
‡Test doses required.

CONCLUSIONS

From the review of these five programs conducted by the ECOG over the past 10 years several conclusions can be drawn. First, EST 1472 confirmed the activity of BCNU in lymphocytic lymphomas. It appears to be as active as cyclophosphamide when both agents are combined with prednisone. Second, toxicity, which is predominantly hematologic, is more severe with the four-drug combination of BCVP than with CVP. Third, BCVP appears to delay relapses during its administration in the lymphocytic lymphomas. Fourth, in Hodgkin's disease, BCVPP appears to be equal to MOPP in the induction phase with less gastrointestinal and central nervous system toxicity.

Hopefully, current studies will clarify, to an even greater extent, the place of BCNU in the treatment of patients with malignant lymphoma.

LIST OF COMPOUNDS

Adriamycin: NSC-123127; CAS reg. No. 23214–92–8

BCNU: NSC-409962; CAS reg. No. 154–93–8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-

BCVP = BCNU, cyclophosphamide, vincristine, and prednisone

BCVPP = BCNU, cyclophosphamide, vinblastine, procarbazine, and prednisone

Bleomycin: NSC-125066; [2,4′-bithiazole]-4-carboxylic acid, 2′-(2-aminoethyl)-, monohydrate

BP = BCNU and prednisone

Chlorambucil: NSC-3088; CAS reg. No. 305–03–3; butyric acid, 4-[*p*-[bis(2-chloroethyl)amino]phenyl]-

COPA = cyclophosphamide, vincristine (Oncovin), prednisone, and adriamycin

COPB = cyclophosphamide, vincristine (Oncovin), prednisone, and bleomycin

COPP = cyclophosphamide, vincristine (Oncovin), prednisone, and procarbazine

CP = cyclophosphamide and prednisone

CPOB = cyclophosphamide, prednisone, vincristine (Oncovin), and bleomycin

CVP = cyclophosphamide, vincristine, and prednisone

Mechlorethamine: NSC-762; CAS reg. No. 55–86–7; diethylamine, 2,2′-dichloro-*N*-methyl-, hydrochloride

MOPP = mechlorethamine, vincristine (Oncovin), prednisone, and procarbazine

Prednisone: NSC-10023; CAS reg. No. 53–03–2; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-

Procarbazine: NSC-77213; CAS reg. No. 491–88–3; *p*-toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, monohydrochloride

Vinblastine: NSC-49842; CAS reg. No. 6449–03–2; vincaleukoblastine, sulfate (1:1), monohydrate

Vincristine: NSC-67574; CAS reg. No. 2068–78–2; leurocristine, sulfate (1:1)

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Nitrosoureas in the Management of Disseminated Malignant Melanoma[1]

David L. Ahmann[2,*]

SUMMARY

Three of the nitrosoureas, BCNU, CCNU, and methyl-CCNU, have displayed significant antitumor activity in patients with disseminated malignant melanoma with none clearly superior to the others, although direct comparison in clinical trials was not accomplished. Response rates to various combinations of these nitrosoureas seem a bit higher, but not dramatically so, when compared to the single-agent response rates, although again this cannot be stated with certainty since direct comparisons were not accomplished. There appears to be no therapeutic advantage to the utilization of any one of the nitrosoureas over the others, but oral administration, as is available for CCNU and methyl-CCNU, is a distinct advantage to the patient and to the clinician. The response rates are, for the most part, rather consistent for the various studies reported in this paper, and these response rates leave a great deal of room for improvement. Secondary therapy utilizing the nitrosoureas was singularly unsuccessful in most of the reported clinical trials. It is refreshing, however, to have agents which have shown activity, although modest, in this most refractory of neoplasms.

[Cancer Treat Rep 60:747–751, 1976]

The malignant melanoma is a relatively uncommon tumor but because of its ability to disseminate widely it has occupied the interest of investigative oncology for some time. Its relative resistance to chemotherapeutic agents has resulted in multiple clinical trials of newer agents as they become available for investigative use. The nitrosoureas represent a class of drugs with demonstrated clinical activity in patients with disseminated malignant melanoma. This paper will attempt to review both the reported experience with these agents and also our experience in consecutive clinical trials. For the most part the studies represent controlled clinical trials in which investigators demanded objective evidence, ie, measurable tumor decrease, of clinical response. In many reports, however, the extent of involvement was not well defined. While the extent of involvement may or may not influence response rates, it certainly does influence survival. The clinical activity of the various nitrosoureas reported seems reasonably consistent between many of the clinical trials. There are exceptions, however, which are undoubtedly related to the extent of disease at the start of treatment and to previous drug exposure in many of the patients. The toxicity of the nitrosoureas has been well discussed in previous presentations and thus will not be referred to here.

Several authors have reported the mean survival of patients with disseminated malignant melanoma from the start of systemic chemotherapy until death, impressively demonstrating the rather rapid clinical course of this disease. In two clinical trials Ahmann et al (1,2) found survival to be relatively short with a mean of 16–18 weeks for all patients entered into first-drug trials with chemotherapeutic agents, including both responders and nonresponders. Understandably, secondary therapy is associated with an even shorter survival with a mean of 8–14 weeks. Luce (3) and Einhorn et al (4) have confirmed these observations with similar survival experiences.

BCNU

BCNU was the first of the nitrosoureas available for clinical trial in patients with disseminated malig-

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nant melanoma. As the other orally administered nitrosoureas became available for clinical trial, they began to limit somewhat the BCNU experience as a single agent. Dose regimens for BCNU alone have varied, but 100 mg/m² on 3 consecutive days seems to be fairly well tolerated. Reported clinical trials by Hill et al (5) and Luce (3) with larger series were consistent, with 16% and 18% objective response rates reported respectively. Broder and Carter[3] summarized the clinical trials with BCNU alone, reporting a 20% level of activity.

CCNU

CCNU, the next nitrosourea available for clinical trial, was able to be administered orally. As a single agent, CCNU has usually been given at a dose of 130 mg/m² on Day 1. Table 1 reviews four clinical trials reported by Hoogstraten et al (6), Cruz et al (7), Pugh,[4] and Ahmann et al (9), demonstrating response rates varying from 5% to 26%. Wasserman et al (9), in a recent summary of CCNU activity in many of the disseminated malignancies, reported an overall response rate of 13% in patients with disseminated malignant melanoma. Mayo Clinic experience (1), representing a randomized clinical trial, contrasted the activity of CCNU versus the combination of DTIC and vincristine. A 5% response rate was observed with CCNU as shown in table 1. The dose of CCNU employed in this study was 130 mg/m² while DTIC was given at a dose of 315 mg/m² daily on each of 5 successive days with vincristine being given at 1.4 mg/m² (not to exceed 2.0 mg) on Days 1 and 5 of the treatment cycle. Secondary chemotherapeutic activity in this clinical trial was convincingly low with this agent as with the other nitrosoureas in this disease.

Methyl-CCNU

Because of its superior activity in the B16 melanoma system (10) and its ability to be given orally, methyl-CCNU was enthusiastically welcomed for clinical trial by investigators treating patients with disseminated malignant melanoma. Table 1 shows the results of many of the larger clinical trials reported. Young et al (11), Costanza et al (12), Tranum et al (13), and Ahmann et al (14) reported activity varying from 0 to 26%. In a recent summary article of methyl-CCNU activity in various malignancies, Wasserman et al (15) reported an overall 12% response rate in this disease. The Mayo Clinic experience (14) (table 1) was accumulated in a randomized clinical trial in which methyl-CCNU given as a single dose (225 mg/m²) administered orally was contrasted with DTIC and vincristine. The dose of DTIC was 325 mg/m² on each of 5 successive days and vincristine was given at a dose of 1.4 mg/m² (not to exceed 2.0 mg) on Days 1 and 5 of the cycle. In this particular clinical trial, methyl-CCNU displayed a response rate (26%) quite comparable to that of the combination of DTIC and vincristine (14). Methyl-CCNU doses have varied in the trials reported above from 150 to 250 mg/m² given orally on Day 1. A single oral dose of 225 mg/m² was clinically well tolerated. The toxicity of the Mayo Clinic trial at this dose level was tolerable and a bit more myelosuppressive than the combination of DTIC and vincristine, but the gastrointestinal side effects were much less, both quantitatively and qualitatively.

[3]Broder LE, and Carter SK. Clinical brochure: 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) NSC-409962. Prepared by the NCI, Bethesda, Md, Sept 1970.

[4]Pugh R, Jacobs E, Bateman J, et al. As quoted by Wasserman et al (9).

TABLE 1.—Results of treatment with the nitrosoureas alone and in combination: disseminated malignant melanoma

Drug(s)	Investigator and ref No.	No. of responses/ No. of patients evaluable	Response rate (%)
CCNU	Hoogstraten (6)	3/17	18
	Cruz (7)	7/27	26
	Pugh (footnote 4)	2/48	4
	Ahmann (8)	1/19	5
	Wasserman (9) (data collected from several sources)	17/136	13
Methyl-CCNU	Young (11)	6/28	21
	Costanza (12)	2/23	9
	Tranum (13)	0/15	0
	Ahmann (14)	5/19	26
	Wasserman (15) (data collected from several sources)	15/124	12

TABLE 1.—Results of treatment with the nitrosoureas alone and in combination: disseminated malignant melanoma—Continued

Drug(s)	Investigator and ref No.	No. of responses/ No. of patients evaluable	Response rate (%)
Methyl-CCNU with—			
DTIC	Ahmann*	6/21	28
DTIC	Costanza (12)	6/26	23
Cyclophosphamide	Ahmann (1)	1/20	5
Cyclophosphamide	Wasserman (15)	3/12	25
Cyclophosphamide + vincristine + bleomycin	Livingston (24)	7/41	17
		23/120	19
BCNU with—			
Vincristine	Moon (16)	9/20	45
Vincristine	Broder (footnote 3)	16/50	32
Vincristine	Moon (17)	8/51	16
Vincristine	Primack (18)	2/16	13
DTIC	Costanza (19)	12/61	19
Vincristine + DTIC	Einhorn (4)	20/106	19
Vincristine + DTIC	Luce (20)	12/27	44
Vincristine + DTIC	Cohen (21)	10/16	63
Vincristine + DTIC	Broder (footnote 3)	15/56	27
Hydroxyurea + DTIC	Costanzi (22)	24/89	27
Hydroxyurea + DTIC + vincristine	Costanzi (22)	47/89	30
Procarbazine + DTIC + vincristine	van Dyk (23)	7/25	28
		162/606	27
CCNU vs DTIC + vincristine	Mayo Clinic		
DTIC + vincristine			
Primary		4/18	
Secondary		1/11	
		5/29	
CCNU			
Primary		1/19	
Secondary		0/7	
		1/26	
Methyl-CCNU vs DTIC + vincristine	Mayo Clinic		
DTIC + vincristine			
Primary		4/19	
Secondary		0/8	
		4/27	
Methyl-CCNU			
Primary		5/19	
Secondary		0/11	
		5/30	

*Ahmann DL, Bisel HF, Edmonson JH, et al. A clinical trial evaluating adriamycin (NSC 123127) and a combination of meCCNU (NSC 95441) and DTIC (NSC 45388) in patients with disseminated malignant melanoma. Submitted for publication.

Combination Chemotherapy

BCNU activity has been exploited in a number of clinical trials and has been utilized in various combinations with other active agents, particularly DTIC and vincristine. Table 1 displays the results of BCNU with vincristine (the most often reported combination); BCNU with DTIC; BCNU with vincristine and DTIC; BCNU with vincristine, DTIC, and hydroxyurea; and BCNU with vincristine, DTIC, and procarbazine. Response rates have varied between 13% and 63%. The mean response rate in the studies reported is 27% (162 of 606 patients), somewhat better than that resulting from BCNU used as a single agent in this disease. Again, as van Dyk and Falkson have noted (23), patients with this disease who have previously been exposed to chemotherapy failed to respond even after polychemotherapy.

Methyl-CCNU has also been used in combination with the following chemotherapeutic agents in clinical trials: DTIC; cyclophosphamide; and cyclophosphamide plus vincristine and bleomycin (table 1). Response rates varied from 5% to 28%. The mean combination response rate was 19% (23 of 120 patients), somewhat better than that reported for methyl-CCNU used as a single agent.

LIST OF COMPOUNDS

BCNU: NSC-409962; CAS reg. No. 154–93–8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-

Bleomycin: NSC-125066; [2,4′-bithiazole]-4-carboxylic acid, 2′-(2-aminoethyl)-, monohydrate

CCNU: NSC-79037; CAS reg. No. 13010–47–4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-

Cyclophosphamide: NSC-26271; CAS reg. No. 6055–19–2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate

DTIC: NSC-45388; CAS reg. No. 4342–03–4; imidazole-4-carboxamide, 5-(3,3-dimethyl-1-triazeno)-

Hydroxyurea: NSC-32065; CAS reg. No. 127–07–1

Methyl-CCNU: NSC-95441; CAS reg. No. 33073–59–5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-

Procarbazine: NSC-77213; CAS reg. No. 491–88–3; *p*-toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, monohydrochloride

Vincristine: NSC-67574; CAS reg. No. 2068–78–2; leurocristine, sulfate (1:1)

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Nitrosoureas as Single Agents in the Treatment of Pulmonary Cancer[1]

Julius Wolf[2,*]

The studies to be described in this report were performed by the Veterans' Administration Cancer Group (VALG) as part of its series of controlled clinical trials of the treatment of patients with inoperable pulmonary cancer. All the patients were men, and except in one trial of BCNU[3] versus radiotherapy, all were categorized as extensive-disease patients. This is a term that was first used by the VALG and has been used extensively by other groups. Extensive-disease patients are stage III with T3, N1 or N2, and generally M1 disease, although there may be a small number of patients with no distant metastases (M0). Limited-disease patients are those with M0 disease but are still stage III with the exception of a small number of patients (< 2%) who are not candidates for surgery because of other medical conditions.

Patients were randomized between several treatment regimens in each of the trials. Since cyclophosphamide[4] was somewhat superior to placebo in previous studies, the other drugs were compared against it as the standard regimen. Patients were stratified by performance status (PS) (the Karnofsky scale) and previous treatment before randomization. The results are analyzed by cell type and PS.

The doses used in the various clinical trials to be reported are listed in table 1. They were modified for toxicity according to predetermined guides.

Figure 1 shows the log-survival experience comparing cyclophosphamide against BCNU. Although there is no significant difference between the two drugs, cyclophosphamide produces a slightly longer survival. Figure 2 similarly shows the log-survival curve of a study comparing BCNU with radiotherapy in patients with limited disease. The two curves were superimposable. Figure 3 is a comparison of cyclophosphamide, BCNU, CCNU, and methyl-CCNU[5] in patients with an initial PS rating of 50–70. Cyclophosphamide-treated patients appear to have had a better survival than those treated with CCNU, with the BCNU- and methyl-CCNU-treated groups showing intermediate survival curves.

The results are practically the same for those patients with an initial PS rating of 80–90, with the BCNU-treated group having the poorest survival and the CCNU- and methyl-CCNU-treated patients' survival falling between that of cyclophosphamide and BCNU.

TABLE 1.—Doses used in various clinical studies

Drug	Dose (mg/m^2)	Schedule
Cyclophosphamide	1100	q3wks
BCNU	250	q6wks
CCNU	130	q6wks
Methyl-CCNU	220	q6wks

Significant differences between the survival of patients treated with one of the nitrosoureas and our historical placebo experience were noted only for CCNU in small cell carcinoma and methyl-CCNU in all cell types (table 2). It should be noted, however, that in no case was there any significant difference in favor of the nitrosoureas over cyclophosphamide.

Toxic effects reported for cyclophosphamide are listed in table 3. Three percent of the patients had severe or life-threatening gastrointestinal toxicity and 9% had severe hematologic side effects. In no case did a patient die of toxicity. Table 3 also indicates that the toxicity reported for BCNU was significantly less than for cyclophosphamide. The toxicity for CCNU (table 3) was approximately the same as that for cyclophosphamide. However, there was one patient who died of pancytopenia. With methyl-CCNU (table 3) there was hematologic toxicity in 20% of the patients, with one death due to staphylococcal pneumonia while the patient experienced severe leukopenia. The blood nadirs were: a

[1]This study was performed by the Veterans' Administration Lung Cancer Group (Julius Wolf, chairman).

[2]Medical Service, Veterans' Administration Hospital (VAH), Bronx, NY.

[3]BCNU: NSC-409962; CAS reg. No. 154–93–8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-.

[4]Cyclophosphamide: NSC-26271; CAS reg. No. 6055–19–2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate.

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[5]CCNU: NSC-79037; CAS reg. No. 13010–47–4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-.

Methyl-CCNU: NSC-95441; CAS reg. No. 33073–59–5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-.

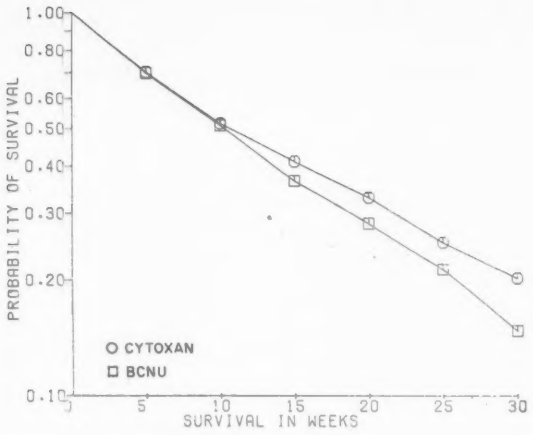

FIGURE 1.—Survival experience of cyclophosphamide [Cytoxan] compared to BCNU.

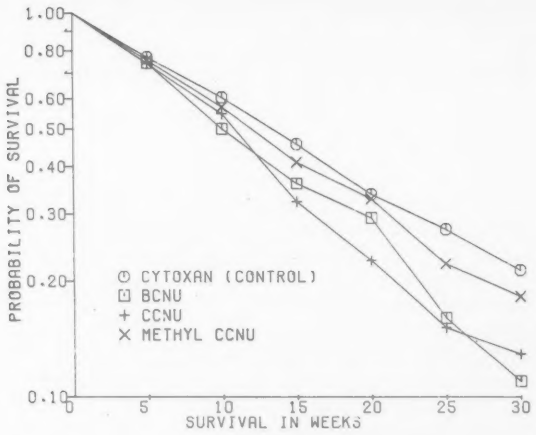

FIGURE 3.—Survival experience with cyclophosphamide [Cytoxan], BCNU, CCNU, and methyl-CCNU in patients with an initial PS rating of 50–70.

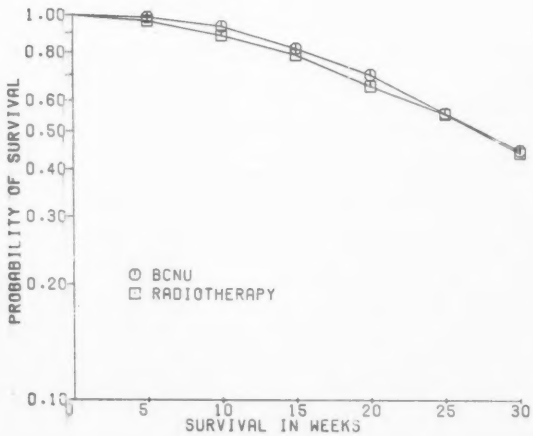

FIGURE 2.—Survival experience of BCNU compared to radiotherapy.

white blood cell count of 1100 cells/mm^3, a platelet count of 32,000 cells/mm^3, and a hematocrit level of 21%; they were all reported 34 days after the last dose of methyl-CCNU (132 days after the study was started). The patient died 30 days after the reported nadirs.

The median survivals in weeks for the patients treated with the nitrosoureas (categorized by PS and cell type) are inferior to those for the patients treated with cyclophosphamide, except for the adenocarcinoma group treated with methyl-CCNU (table 4).

The comparative trials of the three nitrosoureas against cyclophosphamide can be summarized by stating that none of them as individual agents were better than cyclophosphamide; BCNU was probably the worst and methyl-CCNU was somewhat better than CCNU.

TABLE 2.—Significance (at 5% level) of the nitrosourea survival vs placebo by cell type*

	Cell type†				
Nitrosourea	Squamous cell carcinoma	Small cell carcinoma	Adenocarcinoma	Large cell carcinoma	Overall
BCNU	No‡ (35)	No (19)	No‡ (16)	No (10)	No‡ (80)
CCNU	No (77)	Yes (43)	No‡ (41)	No‡ (32)	No (193)
Methyl-CCNU	No (46)	No (25)	No (32)	No (35)	Yes (138)

*Based on the log failure rate, adjusted for initial PS.
†No. of deaths in parentheses.
‡Adjusted failure rate is worse than that for placebo.

TABLE 3.—Toxic effects of cyclophosphamide compared to CCNU, BCNU, and methyl-CCNU

Drug*	No. of patients with—													
	Pain	Nausea	Vomiting	Diarrhea	Gastro-intes-tinal	Bleeding	Skin	Central nervous system	Respira-tory	Genito-urinary	Hemato-logic	Liver	Oral	Other
Cyclophosphamide: grade of toxicity														
0	87	38	45	95	153	96	173	164	94	170	97	173		156
1	10	60	56	5	24	2	7	16	6	10	70	8		10
2	3	2	0	0	5	0	1	1	0	1	16	0		1
1 + 2	13	62	56	5	29	2	8	17	6	11	86	8		11
% with grade 2	3	2	0	0	3	0	1	1	0	1	9	0		1
BCNU: grade of toxicity														
0					50			79		77	35	80	78	72
1					29			0		2	38	0	2	6
2					1			1		1	7	0	0	2
1 + 2					30			1		3	45	0	2	8
% with grade 2					1			1		1	9	0	0	2
CCNU: grade of toxicity														
0	79	52	63	87	153	84	176	170	83	176	87	172		164
1	9	37	26	2	23	4	3	8	5	3	78	4		3
2	1	0	0	0	4	0	0	0	1	1	17	2		0
1 + 2	10	37	26	2	27	4	3	8	6	4	95	6		3
% with grade 2	1	0	0	0	2	0	0	0	1	1	9	1		0
Methyl-CCNU: grade of toxicity														
0	76	50	61	80	150	75	175	171	76	175	74	174		164
1	4	34	23	4	24	5	5	6	7	4	70	4		4
2	3	0	0	0	3	3	1	3	0	2	36	1		2
1 + 2	7	34	23	4	27	8	6	9	7	6	106	5		6
% with grade 2	3	0	0	0	2	4	1	2	0	1	20	1		1

*0 = none; 1 = mild/moderate; 2 = severe/life threatening.

TABLE 4.—Median survivals (wks) for patients categorized by PS and cell type

Drug	PS		Cell type		
	50–70	80–90	Squamous cell carcinoma	Small cell carcinoma	Adenocarcinoma
Cyclophosphamide	13.4	24.0	14.7	14.5	11.9
BCNU	10.0	15.0	20.7	11.5	9.2
CCNU	10.9	21.0	13.2	9.8	13.2
Methyl-CCNU	12.0	19.9	13.2	11.3	14.1

Nitrosourea Combinations in Lung Cancer

Robert B. Livingston[1,*]

SUMMARY

BCNU, CCNU, and methyl-CCNU have undergone extensive trial in multiple-drug combinations for bronchogenic carcinoma. The addition of a nitrosourea appears to be an improvement over cyclophosphamide used alone in oat cell carcinoma and over the two-drug combination of cyclophosphamide and methotrexate in both adenocarcinoma of the lung and oat cell disease. Encouraging response rates have been seen in squamous lung cancer with multiple-drug combinations of a nitrosourea, an alkylating agent, vincristine, and bleomycin with or without adriamycin. The nitrosoureas have been easily incorporated, at reduced doses, into multiple-drug regimens with cumulative myelosuppression seen only when the interval between nitrosourea doses is < 6 weeks. Conclusions about the ultimate role of these compounds in lung cancer treatment must await (*a*) comparative trials of combinations with and without a nitrosourea, and (*b*) further exploration of new approaches to increase their therapeutic index.

[Cancer Treat Rep 60:757–760, 1976]

The nitrosoureas have been extensively evaluated as single agents in lung cancer with overall response rates of 3%–23% depending on cell type and the individual agent used (1). Uncontrolled, cumulative data suggest some advantage for CCNU and methyl-CCNU in squamous carcinoma (14% versus 3% reported response rate for BCNU), large cell undifferentiated carcinoma (about 20% versus 7% for BCNU), and possibly adenocarcinoma (about 10% versus 0 for BCNU). In small cell undifferentiated (oat cell) tumors, response rates of 10%–20% have been reported for these drugs used as single agents with an apparent survival advantage for CCNU over the others in a prospective study.[2] In spite of their relatively unexciting activity as single agents, the nitrosoureas have been extensively combined with other agents because of their ease of administration (usually one dose every 4–8 weeks) and their unique delayed onset of bone marrow toxicity.

One of the earliest of these combination studies was a broad, phase II evaluation of methyl-CCNU and cyclophosphamide used as a two-drug combination at the M.D. Anderson Hospital (2) based on experimental evidence of synergism for these two in animal tumor systems (3). Although the overall result was disappointing (three responses among 19 patients with lung cancer), a tolerated dose and schedule were defined by this study, and the drugs (methyl-CCNU and cyclophosphamide) were subsequently combined with vincristine and bleomycin in a four-drug program (COMB) (4). Using three different schedules which varied the dose and timing of methyl-CCNU and cyclophosphamide only slightly (100 mg/m^2 of methyl-CCNU every 4–6 weeks, and 800–1000 mg/m^2 of cyclophosphamide every 4–6 weeks), COMB elicited partial responses in 33% of 33 patients with squamous carcinoma, 11% of 28 patients with adenocarcinoma, and 87% of 15 patients with oat cell carcinoma of the lung (two complete responses). A subsequent study of the same drugs was done by the Western Cancer Study Group (5) using approximately half the dose of the myelosuppressive components and giving vincristine and bleomycin once per course rather than once per week. Partial responses occurred in five of 28 squamous and nine of 15 oat cell patients. Both the M.D. Anderson Hospital and the Western Cancer

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[2]Wolf J. Unpublished data of the Veterans' Administration Lung Cancer Study Group.

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Study Group results demonstrated significantly longer survival for responding oat cell patients versus nonresponders.

The COMB studies in turn served as the progenitor of another nitrosourea combination employed at the M.D. Anderson Hospital and the University of Indiana for patients with extensive squamous carcinoma of the lung: bleomycin, adriamycin, CCNU, vincristine (Oncovin), and mechlorethamine (nitrogen mustard) (BACON) (6). CCNU was initially given in this program at "half dose," or 65 mg/m², every 4 weeks in combination with 40 mg/m² of adriamycin and 8 mg/m² of mechlorethamine. Cumulative myelosuppression was observed with this regimen and led to a modification in which the CCNU dose was given on Day 1 and then every 8 weeks. In this pilot study, the overall response rate to the BACON regimen was 42% in 50 patients with extensive squamous bronchogenic carcinoma. Median survival from treatment onset was not markedly improved (16 weeks), but responding and stable patients had significantly superior survival times when compared to nonresponders (26 versus 9 weeks, $P = 0.002$). These encouraging initial results led to the current Southwest Oncology Group study which compares two nitrosourea-containing combinations: BACON and NAC (mechlorethamine [nitrogen mustard], adriamycin, and CCNU), a similar three-drug combination from which vincristine and bleomycin have been deleted. It is too early for a definitive analysis of the results of this study.

A variety of two-drug combinations using nitrosoureas have been explored in lung cancer. Curtis (7) studied BCNU and cyclophosphamide (100 and 1000 mg/m², respectively) on an every-3-week schedule and saw objective tumor regression in 14 of 25 (56%) oat cell, one of five large cell, one of 14 squamous cell, and two of 12 adenocarcinoma patients. As with the every-4-week CCNU schedule in BACON, cumulative myelosuppression was observed which necessitated modification of the regimen, in this case a dose reduction rather than a more widely spaced schedule. Stolinsky et al (8) studied 100–150 mg/m² of BCNU over 2 days, repeated every 4 weeks, and 1 mg/m² of vincristine every 2 weeks in a broad phase II trial of solid tumor patients, many of whom had received prior chemotherapy. Tumor regression of > 50% was seen in one of eight oat cell, one of nine "anaplastic," none of six squamous cell, and one of 12 adenocarcinoma of the lung patients.

The combination of CCNU plus cyclophosphamide has been used by the Eastern Cooperative Oncology Group in oat cell carcinoma (9) and by the Veterans' Administration Lung Cancer Study Group (10) in all cell types of bronchogenic carcinoma with extensive disease. Both groups have reported statistically significant superiority for the nitrosourea-containing combination over cyclophosphamide alone in randomized, prospective trials, based on a preliminary analysis of the data. Final response rates and survival figures are still unpublished.

Selawry et al (11) and Hansen et al[3] compared a two-drug combination of cyclophosphamide and methotrexate to a three-drug combination containing these two drugs plus CCNU; in the two-drug combination, cyclophosphamide was given at 1100 mg/m² every 3 weeks and methotrexate was given at 20 mg/m² twice weekly; in the three-drug combination, cyclophosphamide was reduced to 500 mg/m² every 3 weeks, methotrexate to 10 mg/m² twice weekly, and CCNU was given at 50 mg/m² every 6 weeks. The nitrosourea-containing three-drug combination was significantly superior for patients with adenocarcinoma of the lung, both in response rate and survival; it was slightly superior for oat cell patients (final response rates and survival data await publication).

Ansfield (12) has reported a four-drug combination in 39 patients with extensive bronchogenic carcinoma: CCNU (100 mg/m² every 7 weeks); dibromodulcitol (12 mg/kg weekly × 3, repeated every 7 weeks); methotrexate (25 mg weekly × 3, repeated every 7 weeks); and vincristine (1 mg/week × 4, then 0.5 mg every other week). Response rates were not reported, although "most patients with evaluable lung lesions showed significant regressions." However, "liver, brain, or osseous metastases failed to respond."

Pouillart et al (13) administered a three-drug combination of vincristine, CCNU, and 5-fluorouracil (5-FU) to 16 patients with lung cancer, 15 of whom had a squamous histologic diagnosis. Vincristine was given at a dose of 1 mg/m² iv on Days 1 and 2, CCNU at 60 mg/m² orally on Days 3 and 4 (120 mg/m² total dose), and 5-FU at 500 mg/m² over 90 minutes on Days 3, 4, and 5 of each treatment cycle. The cycles were repeated at 4–7-week intervals, depending on evidence of hematologic recovery. The combination was given in this sequential fashion, based on the principle that vincristine may partially recruit tumor cells into a more sensitive phase of the cell cycle (14) and/or potentiate the effects of the subsequently administered "executor" agents by nonspecific, membrane-induced alteration in their pharmacodynamics (15). Pouillart and his co-workers reported a > 50% regression of tumor in nine of 16 patients (eight of 15 squamous), with two complete

[3]Hansen H, Selawry O, Simon R, et al. Combination chemotherapy of advanced lung cancer. A randomized trial. Submitted to *Cancer*, 1975.

remissions. These preliminary results are exciting and require further confirmation.

It is not yet possible to draw definitive conclusions about the role of nitrosoureas in combination-therapy approaches to lung cancer. In oat cell carcinoma, results from three studies suggest an advantage for nitrosourea-containing combinations over cyclophosphamide alone or cyclophosphamide plus methotrexate. Yet none of these results appear superior to those achieved with other combinations containing adriamycin but no nitrosourea (16,17). Comparative trials, and also perhaps studies involving both adriamycin and a nitrosourea, will be necessary to decide whether they form a part of the "best" treatment regimens for oat cell. In squamous carcinoma, final analysis of BACON versus NAC and of the Veterans' Administration Lung Cancer Study Group combination of adriamycin and CCNU will shed light but will still not entirely resolve the issue of the relative activity of component parts. It is clear that adriamycin is more effective than the nitrosoureas as a single drug (18), at least in previously untreated squamous disease. Finally, the encouraging results of Hansen and his co-workers in pulmonary adenocarcinoma, in which a nitrosourea-containing combination appeared superior, must be balanced against the poorer results with COMB and the apparent inactivity of these drugs used singly against this histologic type.

It is possible that the therapeutic index of nitrosoureas could be improved by (*a*) administration of lower doses more frequently (19); (*b*) prior administration of vincristine (13); (*c*) administration of prior or concomitant caffeine, hydroxyurea, or phenformin (20,21); or (*d*) attempts to alter microsomal metabolism of these compounds (22). Perhaps more promising are the attempts to synthesize similar compounds which preserve antitumor effect but lack significant myelosuppression (eg, chlorozotocin [23]). Streptozotocin, the original nonmyelosuppressive nitrosourea to receive clinical trial, appears to lack significant activity in bronchogenic carcinoma (24). Unless developments in one or more of these areas bear fruit, it appears that the role of the nitrosoureas in the treatment of lung cancer will remain much less prominent than animal studies (3) had indicated might be the case.

LIST OF COMPOUNDS

Adriamycin: NSC-123127; CAS reg. No. 23214-92-8

BACON = bleomycin, adriamycin, CCNU, vincristine (Oncovin), and mechlorethamine (nitrogen mustard)

BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-

Bleomycin: NSC-125066; [2,4′-bithiazole]-4-carboxylic acid, 2′-(2-aminoethyl)-, monohydrate

CCNU: NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-

Chlorozotocin: NSC-178248; D-glucopyranose, 2-[[[(2-chloroethyl)nitrosoamino]carbonyl]amino]-2-deoxy-.

COMB = cyclophosphamide, vincristine (Oncovin), methyl-CCNU, and bleomycin

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate

5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8

Mechlorethamine: NSC-762; CAS reg. No. 55-86-7; diethylamine, 2,2′-dichloro-*N*-methyl-, hydrochloride

Methotrexate: NSC-740; CAS reg. No. 59-05-2; glutamic acid, *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-

Methyl-CCNU: NSC-95441; CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-

NAC = mechlorethamine (nitrogen mustard), adriamycin, and CCNU

Streptozotocin: NSC-85998

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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BCNU (NSC-409962) in the Treatment of Advanced Hodgkin's Disease: Its Role in Remission Induction and Maintenance[1]

Tom Anderson,* Vincent T. DeVita, and Robert C. Young[2]

SUMMARY

BCNU is an effective antitumor agent in the treatment of Hodgkin's disease. Forty-seven percent of a group of patients with advanced disease, refractory to combination- or sequential-agent chemotherapy, achieved an objective response to BCNU. Bone marrow toxicity was delayed in onset and was dose related. When incorporated into a maintenance program after remission induction with combination chemotherapy, BCNU appeared to delay, but not ultimately prevent, relapses during a 2-year followup period. Toxic effects associated with BCNU maintenance therapy in this clinical situation were significantly greater than toxic effects associated with no maintenance therapy.

[Cancer Treat Rep 60:761–767, 1976]

With the introduction of effective combination chemotherapeutic regimens, the prognosis of advanced Hodgkin's disease has been drastically altered. The initial work of DeVita et al (1) demonstrated that the combination of mechlorethamine, vincristine, procarbazine, and prednisone (MOPP)[3] could induce long-term remissions in 81% of stage III and IV patients. The median duration of remission was 36 months with 43% of the patients disease free 5 1/2 years after completing a successful remission-induction course of MOPP therapy.

Nevertheless, 19% of all patients with advanced Hodgkin's disease never achieve a CR and 57% of those patients who do achieve a CR subsequently relapse. Because initial clinical trials with BCNU (2,3) had indicated that this new class of compounds had activity against Hodgkin's disease, the NCI Medicine Branch undertook the systematic evaluation of BCNU therapy for Hodgkin's disease patients who had developed resistance to alkylating agents, vinca alkaloids, and radiotherapy (4). After demonstrating the efficacy of BCNU in this resistant patient population, the evaluation of BCNU as a remission maintenance agent was also investigated (5). This report is a review of our experience with BCNU in the management of patients with Hodgkin's disease in these two clinical settings.

BCNU IN HODGKIN'S DISEASE RELAPSE

Patient Selection

Forty-five patients with Hodgkin's disease resistant to alkylating agents, vinca alkaloids, and radiotherapy were treated with BCNU. Seventeen of 45 patients (38%) had failed MOPP combination chemotherapy; 23 of 45 patients (51%) had received intensive involved- or extended-field radiotherapy but had relapsed in nonirradiated areas and subsequently failed single-agent chemotherapeutic regimens; and five of 45 patients (11%) had received single-agent chemotherapy as their primary therapy. Forty-three of 45 patients (96%) had been treated with an alkylating agent; 43 of 45 patients (96%) had also been treated with a vinca alkaloid. Patients were treated when they had evidence of advancing disease resistant to standard therapy. Of

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[1]BCNU: CAS reg. No. 154–93–8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-.

[2]Medicine Branch, Clinical Oncology (CO), Division of Cancer Treatment (DCT), National Cancer Institute (NCI), Bethesda, Md.

[3]Mechlorethamine: NSC-762; CAS reg. No. 55–86–7; diethylamine, 2,2'-dichloro-*N*-methyl-, hydrochloride.

Vincristine: NSC-67574; CAS reg. No. 2068–78–2; leurocristine, sulfate (1:1).

Procarbazine: NSC-77213; CAS reg. No. 491–88–3; *p*-toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, monohydrochloride.

Prednisone: NSC-10023; CAS reg. No. 53–03–2; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-.

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the 45 patients, one (2%) was stage IIIA, four (9%) were stage IIIB, and 40 (89%) were stage IVB at the time BCNU was administered. All patients were considered evaluable once BCNU was administered; none were excluded because of early death or an "inadequate trial."

Drug Administration

In this study BCNU was solubilized in a 10% ethanol:water solution and administered with normal saline over 10–30 minutes. Initially, patients were treated with a dose of 100 mg/m^2; after establishing the safety of this dose, subsequent patients were escalated to higher doses (maximum dose 250 mg/m^2). During the early phase of the trial, drug administration varied between a single iv dose (35 of 45 patients [78%]), a biweekly divided dose (seven of 45 patients [16%]), and a fractionated dose given over 2–3 days (three of 45 patients [7%]). No difference in toxicity was noted between the various regimens.

Patients were carefully observed during the 4 weeks after the initial dose of BCNU. If the patients' lesions grew during this period of time, they were classified as BCNU failures. Patients whose lesions had stabilized or regressed received repeated doses of BCNU at 4–6-week intervals. Seventeen of 45 patients (38%) received a single dose of BCNU, ten (22%) received two doses, and 18 received three or more doses. The maximal cumulative dose was 1800 mg/m^2 administered over 60 weeks.

Response Criteria

CR was defined as complete disappearance of all measurable evidence of disease, all laboratory abnormalities presumably related to the patient's disease, and all subjective symptoms. PR was defined as a ≥50% reduction in the size of all measurable disease and disappearance of all subjective symptoms. Nonresponders were those patients in whom at least one tumor grew while receiving therapy (regardless of any evidence of response in other areas), patients whose symptoms failed to disappear, and patients with an apparent transient objective response lasting < 3 weeks.

Toxic Effects

Rapid infusion of the larger doses of BCNU produced flushing, conjunctival injection, and nausea and vomiting usually within 2 hours of infusion which subsequently lasted several hours. These toxic effects could be partially ameliorated by the prior administration of sedatives and/or antiemetics. A burning sensation at the site of infusion was common and could be minimized by decreasing the infusion rate; no phlebitis was observed.

Bone marrow depression was the major dose-limiting toxic effect and was delayed in its onset, a manifestation now recognized as characteristic of this class of compounds (table 1). Thrombocytopenia (platelet count ≥100,000 cells/mm^3) usually occurred more frequently than leukopenia (white blood cell count ≥4000 cells/mm^3): 71% versus 53%. The thrombocytopenia was also observed somewhat ear-

TABLE 1.—Bone marrow toxicity of BCNU in 45 patients with Hodgkin's disease

Dose (mg/m^2)	No. of patients	No. of patients with— Leukopenia	Thrombocytopenia (cells/mm^3) <100,000	<50,000	Total	Mean day of nadir	Nadir count (× 10^3 cells/mm^3) Mean	Range	Mean interval before recovery (days)
Leukopenia									
100	12	3 (25%)				35	2.4	1.7–3.4	6.0
150	8	5 (62%)				28	1.4	0.9–1.6	15.6
200	15	11 (73%)				31	2.0	0.3–3.6	10.6
250	10	5 (50%)				40	2.5	0.8–3.4	10.2
Totals	45	24 (53%)				34	2.0	0.3–3.6	10.6
Thrombocytopenia									
100	12		2	4	6 (50%)	29	42	9–80	4.0
150	8		2	4	6 (75%)	32	30	7–82	8.8
200	15		6	8	14 (93%)	26	40	9–98	8.8
250	10		2	4	6 (60%)	32	52	17–90	12.5
Totals	45		12 (27%)	20 (44%)	32 (71%)	30	41	7–98	8.5

lier than leukopenia (mean nadir, Day 30 vs Day 34) and was of slightly lesser duration (mean interval before recovery, 8.5 vs 10.6 days). The relationship between the dose and the degree of leukopenia and thrombocytopenia was not striking except for a minor difference in the rate of recovery noted at only the lowest dose (100 mg/m²); this difference was of questionable significance (table 1).

Serial evaluation of hepatic function, utilizing serum enzymes, scans, and in some cases percutaneous biopsy, failed to show a significant degree of hepatic toxicity as had been demonstrated by the animal toxicology screen. Only two patients (4%) had evidence of such toxicity; in one patient, however, this apparent toxicity was a contributory cause of death. Careful evaluation of renal function failed to show any renal toxicity.

Three deaths (7%) could be related to drug toxicity; all three patients had fatal infections associated with an episode of pancytopenia and one of the three also had apparent hepatic toxicity.

Results

Twenty-one of 45 patients (47%) had an objective response and 19 of 45 (42%) had a PR. The mean time to response was 11.8 days (range, 1–60 days) and the median duration of response was 16.5 weeks. Two patients (4%) had CRs lasting 72 and 208 weeks respectively. The responses were frequently dramatic; subjective improvement usually preceded objective response and allowed a majority of the responders to be discharged from the hospital. It was characteristic to see a tumor response before the onset of bone marrow toxicity. Responses were seen at all dose levels; the higher dose levels (200 and 250 mg/m²) had a combined response rate of 56% (14 of 25 patients) compared with a response rate of 45% (nine of 20 patients) at the lower dose levels (100 and 150 mg/m²) (table 2); this difference was not significant at the $P < 0.05$ level.

There were no significant differences in age, sex, stage of disease, or duration of disease between patients who responded to BCNU and those who did not. Although patients who had previously achieved prolonged remissions with multiple-drug therapy early in the course of their disease more frequently responded to BCNU than those who had not responded to previous agents, two of 21 responders (10%) had failed all previous chemotherapeutic regimens. In general, there was no characteristic response to previous therapy that predicted a response to BCNU (table 2).

BCNU AS MAINTENANCE CHEMOTHERAPY FOR ADVANCED HODGKIN'S DISEASE IN REMISSION

In July 1967 a clinical trial was initiated to examine the effects of maintenance chemotherapy after induction of a CR with MOPP in patients with advanced Hodgkin's disease. This trial was prompted by the observation that 57% of the patients achieving a CR subsequently relapsed. Because the previous trial had demonstrated a high degree of activity in relapsing patients, BCNU was included in the maintenance trial. After entering CR, patients were randomly allocated to one of three regimens: no additional therapy, intermittent MOPP therapy, or intermittent BCNU therapy.

Patients and Methods

Between 1967 and 1972, 86 patients with advanced Hodgkin's disease were given MOPP therapy as previously described. All patients had biopsy-proven Hodgkin's disease confirmed by the Laboratory of Pathology, NCI, and subclassified by the histologic criteria of Lukes and Butler (6). Clinical staging was initially based upon the Rye classification; subsequently, all patients were reclassified according to the newly adopted Ann Arbor classification (7). Clinical staging procedures were generally those recommended by the Ann Arbor Symposium. All patients had a metastatic bone series, bipedal lymphangiography, and liver-spleen scans. Bone marrow aspirations and biopsies were routinely done. Initially, percutaneous liver biopsies were performed based

TABLE 2.—BCNU response in relation to previous chemotherapy

	BCNU responders (21 patients)		BCNU nonresponders (24 patients)	
	No. of patients—		No. of patients—	
Previous chemotherapy	Treated	Responding	Treated	Responding
Alkylating agents	19	15 (79%)	23	17 (74%)
Vinca alkaloids	20	14 (70%)	23	16 (70%)
Procarbazine	11	8 (73%)	15	11 (73%)

upon clinical suspicion of liver involvement; during the latter portion of the study they were routinely done on all patients. All patients with a negative percutaneous liver biopsy had a peritoneoscopy with four to six biopsies performed under direct visualization. Staging laparotomies were not performed. When patients were reclassified according to the Ann Arbor classification only biopsy-proven organ involvement was accepted for stage IV patients.

Sixty-four of 86 patients (74%) achieved a CR after a minimum of six cycles of MOPP therapy; unlike the original MOPP study, those patients who did not have a CR within six cycles of induction therapy received further cycles of MOPP until CR was achieved. Two additional cycles of therapy were given after CR for consolidation purposes. Usually the patients with stage IV disease had a repeat biopsy of the organ(s) previously involved to document completeness of remission before induction therapy was discontinued. The mean times to CR for the groups receiving no further therapy, intermittent MOPP, or intermittent BCNU were 4.5, 4.1, and 4.0 months respectively. The mean numbers of cycles given for the three groups were 7.1, 6.7, and 6.5 respectively. Twenty-nine percent of the patients subsequently given no further therapy required more than six cycles to complete induction therapy, while 25% of those given intermittent MOPP therapy and 19% of those given BCNU therapy required a similar extension of therapy.

Maintenance Therapy

Of the 64 patients achieving remissions, five were not randomized to the maintenance phase of the study for reasons previously reported (5) and the period of followup on two patients was inadequate for evaluation. Therefore, a total of 57 patients were randomized: 21 patients to no additional therapy, 20 patients to intermittent MOPP therapy, and 16 patients to BCNU therapy. Maintenance MOPP therapy was administered in the same doses used in the induction phase as two consecutive monthly cycles every 3 months for 15 months (a total of ten cycles of maintenance MOPP therapy). Maintenance BCNU therapy was administered at a dose of 200 mg/m^2 every 3 months for 15 months (a total of five cycles of BCNU). Both maintenance regimens were started within 3 months of the completion of induction therapy. The survival was computed from the beginning of induction chemotherapy; the remission duration was calculated from the end of induction chemotherapy. Statistical analyses were performed as previously described (5); the significance was assessed at the 0.05 level unless otherwise stated.

Results

The characteristics of the patients randomized to the maintenance study are shown in table 3. There was a difference in age with the random allocation of younger patients to the group receiving BCNU maintenance therapy; however, the mean age of all three groups was within the usual range of bimodal incidence peaks for Hodgkin's disease. No other differences in patient characteristics or in histologic or staging criteria were noted.

The effect of maintenance therapy on the number of relapses, the time of relapse, and the median

TABLE 3.—Patient characteristics

Characteristic	Therapy		
	No maintenance	Intermittent MOPP	Intermittent BCNU
General			
No. of patients	21	20	16
Mean age (yrs)	34.1	36.8	25.4
Men/women	15/6	12/8	12/4
No. with previous radiotherapy	3	2	3
Histology			
Lymphocyte depleted	2	4	3
Mixed cellularity	11	8	8
Nodular sclerosis	7	7	5
Lymphocyte predominant	1	1	0
Stage			
IIIB	9	5	6
IIIB$_e$	2	2	0
IVA	2	0	2
IVB	8	13	8

TABLE 4.—Followup results

	Therapy		
	No maintenance	Intermittent MOPP	Intermittent BCNU
No. of patients	21	20	16
No. of patients relapsing	5 (24%)	5 (25%)	2 (13%)
Mean time to relapse (mos)	6	25.8	25.5
No. of deaths from Hodgkin's disease	1	1	1
Time to death (mos)	11	35	52
Median duration of remission (mos)	> 48	> 48	> 48

TABLE 5.—Complications of therapy

	Therapy		
Complication	No maintenance	Intermittent MOPP	Intermittent BCNU
Herpes zoster	1	0	3
Pneumonia	0	1	2
Severe bronchitis	0	0	2
Persistent amenorrhea	0	0	1
Chronic leukopenia	0	0	2 (1 with myelofibrosis)
Chronic pancytopenia	0	2 (1 with sepsis)	0
Totals	1	3	10

duration of remission is shown in table 4. The differences in relapse rates were not significant. While the mean time to relapse was significantly shorter for those patients receiving no maintenance therapy than for those patients receiving either form of maintenance, the median time to relapse was not different for any of the three groups. Furthermore, there was no significant difference in the median duration of remission among the three study groups, with a median remission of ≥ 4 years projected for all groups. Essentially, the maintenance therapy only delayed an inevitable relapse in a few patients. The patients who relapsed were retreated with MOPP therapy with the exception of three patients in the MOPP maintenance group. Most of the patients achieved a second remission with MOPP therapy but these were usually transient. The overall survival for all three groups is shown in figure 1.

Complications

The risk of complications and incidence of infection are summarized in table 5. The median followup time was equivalent in each of the randomized groups. Pancytopenia or myelofibrosis was not observed in the group receiving no further therapy after CR induction, and only one patient had a mild localized herpes zoster which resolved spontaneously. In contrast, in both of the maintenance studies two patients developed pancytopenia necessitating modification of maintenance drug dosage. Bacterial infections were more frequent in patients receiving maintenance therapy and this was especially true of those receiving intermittent BCNU therapy ($P = 0.03$).

DISCUSSION

While a significant number of patients with advanced Hodgkin's disease respond to MOPP combination chemotherapy, > 50% of these patients either fail initial induction therapy or subsequently relapse and become resistant to this regimen. BCNU has been investigated in an attempt to identify an agent which can produce meaningful remissions in these patients. Reproducible results from a

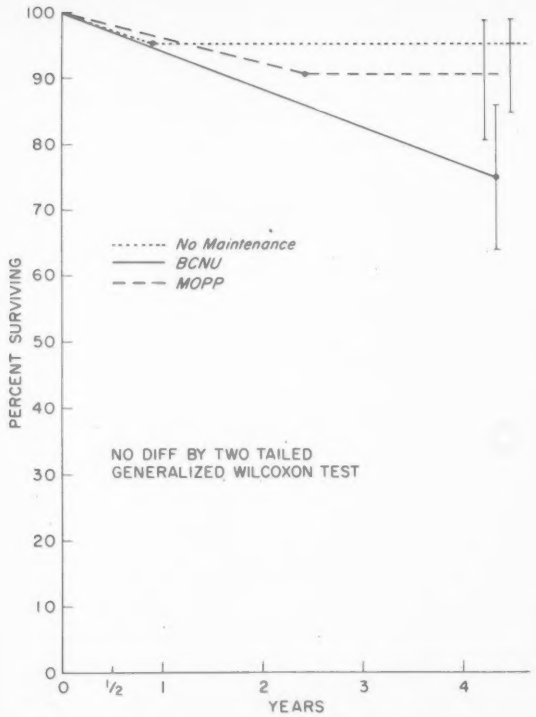

FIGURE 1.—Survival in advanced Hodgkin's disease (3 maintenance arms).

number of studies, in addition to our own experience, now confirm that BCNU or one of its analogs should be considered for all these patients (table 6). A 47% response rate to single-agent chemotherapy, especially after failing previous chemotherapy, is extremely gratifying. While BCNU produces delayed bone marrow toxicity, most patients who respond can be treated on an out-patient basis for significant periods of time.

The ability of BCNU to produce a response in patients resistant to standard alkylating-agent therapy has stimulated investigation into the mechanism of action of this class of compounds. While the end result of treatment with these agents is a depression of DNA synthesis (8,9), and these agents alkylate DNA in vitro (10), other potential mechanisms of antitumor activity are being explored (3). Inhibition of DNA polymerase activity has been demonstrated (11), as has inhibition of RNA and protein synthesis (12,13); details of some of these studies are published in this issue.

Because of its activity in Hodgkin's disease patients who had relapsed, it was hoped that the incorporation of BCNU in a maintenance phase of

TABLE 6.—BCNU response in advanced Hodgkin's disease

No. of evaluable patients	No. of responders	Ref No.
45	21 (47%)	4
31	17 (55%)	2
149	75 (50%)	3
28	10 (36%)	17
29	10 (34%)	*
33	16 (48%)	18
Totals 315	149 (47%)	

*Bateman J. Personal communication, 1974.

an integrated chemotherapeutic regimen would decrease the number of patients who were at risk of relapse after successful remission induction therapy with MOPP. The study was designed to determine whether the addition of a new agent, which was not cross resistant with the agents used during induction therapy, would be more efficacious than intermittent therapy with the original agents used during induction therapy. As shown in table 4, both BCNU and intermittent MOPP maintenance therapy delayed the mean onset of relapse for only a few patients. The relapse rate, the median time to relapse, and the duration of remissions for each of the three groups were similar. When patients were followed for 2+ years, the ultimate relapse rates in both groups were the same as that observed in the control group and, therefore, had no significant effect upon long-term survival (fig 1). Similar conclusions have been drawn from at least two other studies of maintenance therapy in Hodgkin's disease using these and other agents (14,15).[4,5] In addition, it was noted that the number of significant complications was increased in the patients receiving maintenance therapy, particularly BCNU maintenance therapy. Unfortunately, none of the other maintenance studies have reported on infectious complications relating to maintenance therapy.

Our experience shows that long-term survivals free of disease are common in patients with advanced Hodgkin's disease who achieve CR after treatment with combination chemotherapy alone. Maintenance chemotherapy theoretically might decrease the number of patients who subsequently relapse. BCNU was a logical choice for such a program because of its lack of cross-resistance with alkylating agents or with any other agent in the remission-induction regimen. However, our experience shows that although relapse may be delayed in a few patients, ultimate disease-free survival is not altered and toxicity is significantly increased. For

[4]Coltman CA. Personal communication.
[5]Durant JR. Personal communication.

these reasons, neither BCNU nor MOPP combination chemotherapy is being utilized as maintenance-remission therapy at our institution.

Various institutions are investigating the role of BCNU or other nitrosoureas in combination with other agents to determine their roles in induction of initial remission (15,16). The results of such studies have not yet been published, but preliminary results indicate a lack of significant increase in remission duration associated with the addition of BCNU to the induction regimen. Pending the final results of such studies we feel that BCNU is a useful and active agent in patients with Hodgkin's disease, but because of its toxicity should be used for patients who have developed resistance to combination chemotherapy. There is currently no evidence that it is beneficial as a routine remission-maintenance agent.

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Nitrosoureas: Useful Agents for the Treatment of Advanced Gastrointestinal Cancer[1,2]

H. O. Douglass, Jr,[3,*] P. T. Lavin,[4] and C. G. Moertel[5]

SUMMARY

Patients with a wide range of gastrointestinal cancers have been treated with nitrosoureas by the Eastern Cooperative Oncology Group. Methyl-CCNU, CCNU, and streptozotocin have been evaluated as single agents in the treatment of colorectal carcinoma. Methyl-CCNU has had an extensive trial in gastric carcinoma as a single agent and in combination with 5-fluorouracil (5-FU). It has also been used to treat pancreatic carcinoma and, in a few patients, carcinoma of the biliary tract. In gastric cancer it would appear that a synergistic effect on response rates has resulted from the combination of methyl-CCNU and 5-FU. The addition of cyclophosphamide to this combination as an induction agent detracted significantly.

[Cancer Treat Rep 60:769–780, 1976]

Preliminary trials by Schabel demonstrated that BCNU would be more effective against L1210 leukemia than cyclophosphamide. Until that time, cyclophosphamide had been considered the most effective agent in this tumor model. It was then noted that CCNU crossed the blood-brain barrier, permitting control of intracerebral implants of L1210 leukemia (1). Further studies from the same laboratory (2) indicated that methyl-CCNU was the most effective drug found to date against Lewis lung carcinoma, a solid tumor that had been resistant to most other chemotherapeutic agents. These investigators later demonstrated that, in both the L1210 and AKR leukemia systems, nitrosoureas could be used in combination with other agents with less than additive toxicity while showing more than additive tumor response. In addition, activity against a transmissible colonic tumor in BALB/c mice was later demonstrated.

Preliminary studies in man established acceptable dosage schedules for CCNU and, later, for methyl-CCNU.[6] The activity of these drugs was explored by the Eastern Cooperative Oncology Group (ECOG) in patients with gastrointestinal (GI) carcinomas.

PROTOCOLS

The first ECOG trials of nitrosoureas in GI cancer were undertaken comparing CCNU and streptozotocin to procarbazine, 6-thioguanine, and bleomycin in patients with metastatic colorectal carcinoma in a study designed by Dr. John Horton (Natalie Warren Bryant Cancer Center, Tulsa, Okla). A number of patients who were entered in this study had been previously treated with 5-fluorouracil (5-FU).

In 1973, the ECOG activated a new protocol in which the activity of methyl-CCNU was evaluated in both untreated and previously treated patients (fig 1). The inclusion of patients receiving methyl-CCNU either before or after 5-FU therapy in combination with either 6-thioguanine or cyclophosphamide permitted a comparison of the responsiveness

[1]Supported in part by Public Health Service grants CA-12296 and CA-12721 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[2]This report was drawn from studies performed by the Eastern Cooperative Oncology Group (ECOG) (Paul P. Carbone, chairman).

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[6] Comis RL, Broder LE, and Carter SK. Clinical brochure: 1-(2-chlorethyl)-3-(4-methyl-cyclohexyl)-1-nitrosourea (Me-CCNU) NSC-95441. Prepared by the NCI, Bethesda, Md, Aug 1972, 80 pp.

of patients to methyl-CCNU given before or after these two combinations.

The current ECOG protocol (fig 2) for advanced colorectal carcinoma evaluates the possible potentiation of the response rate to 5-FU and methyl-CCNU by combination with other agents which may increase their activity. The combination of 5-FU and methyl-CCNU will be compared to 5-FU alone in patients who are treated with palliative resection for tumors with liver metastases (Duke's D) or with resection with intent to cure in the presence of serosal invasion or lymph node metastases (Duke's B and C).

The activity of methyl-CCNU was explored when the drug was used alone or in combination with 5-FU in the treatment of gastric carcinoma (fig 3). The concept of induction of responses using cyclophosphamide was explored based on experience with hematologic malignancies and on evidence of a lack of cross reactivity between alkylating agents and nitrosoureas. As a result of these studies, both the ECOG and the Gastrointestinal Tumor Study Group (GITSG) are exploring surgical adjuvant chemotherapy using 5-FU and methyl-CCNU.

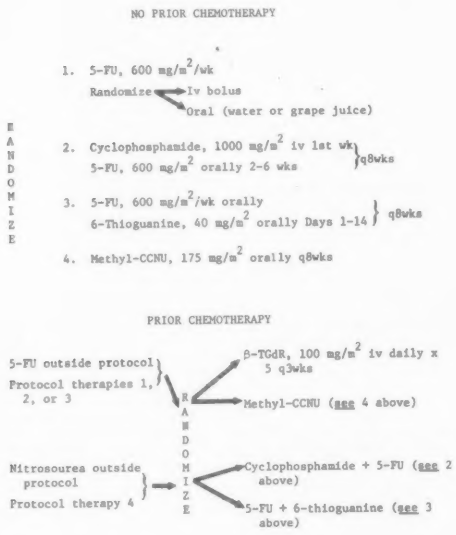

FIGURE 1.—EST-4273. Protocol scheme for the chemotherapy of advanced colorectal carcinoma.

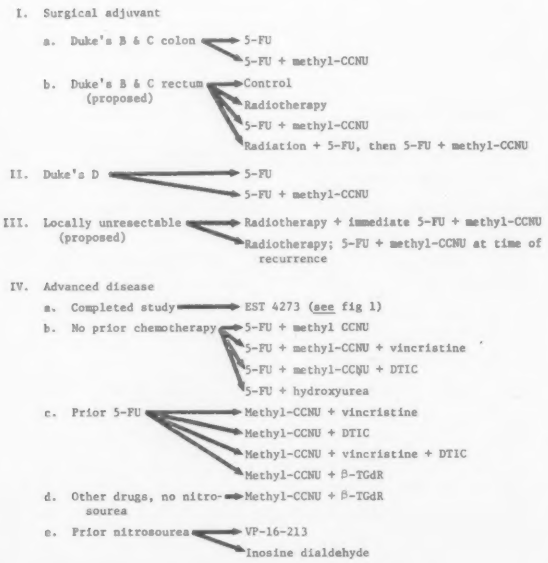

FIGURE 2.—ECOG protocols for various stages of colorectal adenocarcinoma.

RESULTS IN ADVANCED COLORECTAL CANCER

Toxic Effects

Significant toxic effects (rated as severe or greater [table 1] occurred in 17% of the patients treated with CCNU and in 21% of the patients treated with methyl-CCNU (fig 4). These toxic effects were due to very severe vomiting in 9% of the patients treated with methyl-CCNU and due to bleeding secondary to thrombocytopenia in 6%.

Response Rates

In patients who had received no prior chemotherapy (table 2), eight of 50 who were treated with methyl-CCNU showed objective responses (50% or greater reduction in tumor size). Objective responses were seen in six of 20 patients treated with CCNU, but in none of the patients treated with streptozotocin.

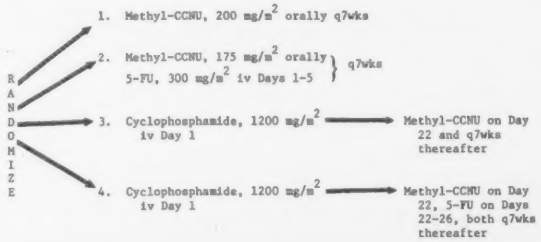
1. Methyl-CCNU, 200 mg/m2 orally q7wks
2. Methyl-CCNU, 175 mg/m2 orally
5-FU, 300 mg/m2 iv Days 1-5
q7wks
RANDOMIZE
3. Cyclophosphamide, 1200 mg/m2 iv Day 1
Methyl-CCNU on Day 22 and q7wks thereafter
4. Cyclophosphamide, 1200 mg/m2 iv Day 1
Methyl-CCNU on Day 22, 5-FU on Days 22-26, both q7wks thereafter

FIGURE 3.—EST-1272. Protocol scheme for the chemotherapy of advanced gastric carcinoma, exploring the concept of cyclophosphamide induction of responses in solid tumors, and the effect of combination of methyl-CCNU with 5-FU.

In patients who had received prior chemotherapy with 5-FU or other agents, 15% (five of 33 patients) showed response to streptozotocin. Objective responses were seen in only 3% of the patients treated with CCNU (one of 40 patients) and in 5% of those treated with methyl-CCNU (three of 64).

Objective responses often did not translate into prolonged survival. Although 15% of the patients who had previous chemotherapy responded to streptozotocin, their mean survival was only 13 weeks. In a group of similar patients treated in the same protocol with procarbazine, a drug with which there were no responders recorded, the median survival was 26 weeks. Compared to a median survival of 20 weeks in all patients who received CCNU (both untreated and previously treated), the median survival of patients treated with procarbazine was 22 weeks.

In a later protocol similar patients treated with methyl-CCNU who had not previously received 5-FU or other agents showed a mean survival of 31 weeks. Those who had been previously treated showed a mean survival of only 21 weeks. There was

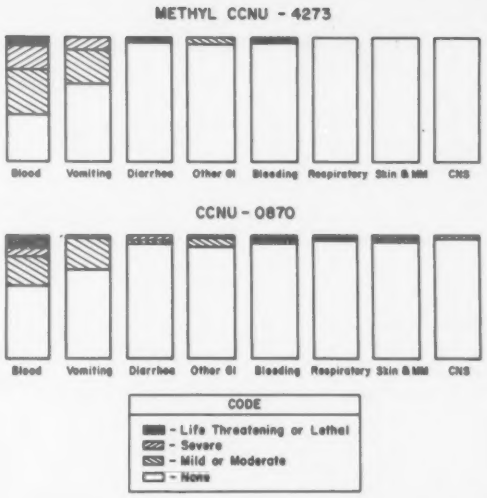

FIGURE 4.—Comparative toxicities of methyl-CCNU and CCNU in advanced colorectal carcinoma. Each bar represents 100% of the patient data analyzed for each toxicity parameter.

TABLE 1.—Summary of ECOG toxicity criteria

Toxic effects	Degree			
	None (0)	Mild-moderate (1–2)	Severe (3)	Life threatening (4)
White blood cell count (cells × 10^3/mm^3)	≥ 4.5	2.0–< 4.5	1.0–< 2	< 1.0
Platelet count (cells × 10^3/mm^3)	≥ 130	50–< 130	25–< 50	< 25
Hemoglobin level (g/100 ml)	≥ 11	Symptomatic	Transfused	
Bleeding		Not debilitating	Debilitating	Hypotensive
Infection		Responsive to treatment	Debilitating	Septic
Blood urea nitrogen level (mg/100 ml)	≤ 20	21–60	> 60	Symptomatic
Bilirubin level (mg/100 ml)	< 1.5	1.5–5	> 5	Hepatic coma
Cardiac toxicity		Atrial premature contraction, ST-T change	Premature ventricular contraction, congestive heart failure	Refractory
Neurotoxicity		Anxiety, headache	Weakness, confusion	Seizures, coma
Vomiting		Controllable	Intractable	
Diarrhea		Dehydration	Bloody	
Stomatitis		Can eat	Cannot eat	

TABLE 2.—Effect of prior chemotherapy with 5-FU and other agents on response and survival of patients with advanced colorectal adenocarcinoma treated with nitrosoureas as single agents (ECOG experience 1970–1975)

Protocol (EST No.)	Drug	Dose (mg/m^2) and schedule	No prior chemotherapy		Prior chemotherapy	
			Mean survival (wks)	No. of responses/ No. of patients evaluable	Mean survival (wks)	No. of responses/ No. of patients evaluable
4273	Methyl-CCNU	175, q8wks	31	8/50 (16%)	21	3/64 (5%)
0870	CCNU	130, q6wks	23	6/20 (30%)	17	1/40 (3%)
0870	Streptozotocin	500, weekly	11	0/17	13	5/33 (15%)

no correlation among patients treated with nitrosoureas between survival and age, sex, or prior radiation therapy.

The analysis of sequential therapies (5-FU with either cyclophosphamide or 6-thioguanine followed, after failure, by methyl-CCNU versus the reverse order of treatment) revealed little difference in the overall time on treatment (table 3). In both groups the time to progression after secondary treatment ranged between 21 and 28 weeks.

Factors Influencing Response

The physical status of a patient was an important determinant of response and survival in patients treated with the nitrosoureas. When "ambulatory" is defined as being out of bed all day and "nonambulatory" is defined as patients who must be in bed for at least a part of their normal waking hours, analysis of physical status becomes more meaningful. Five of 39 patients treated with methyl-CCNU who were ambulatory at the time treatment was initiated showed objective tumor responses. In four of 16 nonambulatory patients there was also an objective tumor response. However, the median survival in patients who were ambulatory was 28 weeks, whereas that of patients who were nonambulatory was only 12 weeks (table 4).

In patients treated in the earlier protocol with CCNU, five of 21 had an objective response and the median survival was also 28 weeks. There were no responders among 32 nonambulatory patients treated with CCNU, and the median survival was only 13 weeks. Although there was little difference in the response rate of ambulatory and nonambulatory patients treated with streptozotocin, the median survival of ambulatory patients was 20 weeks whereas that of nonambulatory patients was 7 weeks.

Is it necessary to have some evidence of toxicity in order to expect results from nitrosoureas? Does the nitrosourea have to be administered at a dose level that is expected to result in some toxicity before a response can be expected? Does increasing the dose of the nitrosourea, thus increasing the degree of toxicity, offer a potential increase in response rate?

Patients who had no evidence of toxicity to nitrosoureas had median survival rates similar to those of patients who showed severe degrees of toxicity. A mild degree of maximum toxicity appeared to be associated with prolonged survival.

At 1 year, the patients who had mild hematologic toxicity were nearly twice as likely to be alive as those who showed either no toxicity or severe toxic-

TABLE 3.—Effect of the order in which therapies are sequenced on the time to progression in patients with advanced colorectal carcinoma (EST No. 4273)

Initial treatment		Subsequent therapy after failure of initial treatment		No. of weeks on protocol-specific therapy
Drugs	Duration of therapy (wks)	Drugs	Duration of therapy (wks)	
Cyclophosphamide + 5-FU	17	Methyl-CCNU	11	28
6-Thioguanine + 5-FU	14	Methyl-CCNU	8	22
Methyl-CCNU	9	Cyclophosphamide + 5-FU	12	21
Methyl-CCNU	13	6-Thioguanine + 5-FU	12	25
All patients treated with methyl-CCNU	16	All patients treated with methyl-CCNU	14	—

TABLE 4.—Response rates and survival of patients with advanced colorectal carcinoma treated with nitrosoureas (1970–1975) (median survival of ambulatory patients was more than twice as long as that of nonambulatory patients)

Protocol (EST No.)	Drug	Ambulatory patients		Nonambulatory patients	
		No. of responses/ No. of patients evaluable	Median survival (wks)	No. of responses/ No. of patients evaluable	Median survival (wks)
4273	Methyl-CCNU	5/39	28	4/16	12
0870	CCNU	5/21	28	0/32	13
0870	Streptozotocin	3/23	20	2/23	7

ity when treated with either CCNU or methyl-CCNU (fig 5). This phenomenon was not dependent on physical status, but was noted among patients who were ambulatory (fig 6*a*) and among those who were nonambulatory (fig 6*b*), although the survival of the nonambulatory patients was markedly inferior. In contrast to hematologic toxicities, there was little difference in the survival of patients who had no toxicity or minimal to mild toxicity of a GI nature related to nausea and vomiting (figs 7*a* and 7*b*). When all toxicities were considered (fig 8), it appeared that a mild to moderate degree of toxicity was necessary to result in a prolongation of survival. When analyzing all toxicities, this was true in both ambulatory and nonambulatory patients (figs 9*a* and 9*b*).

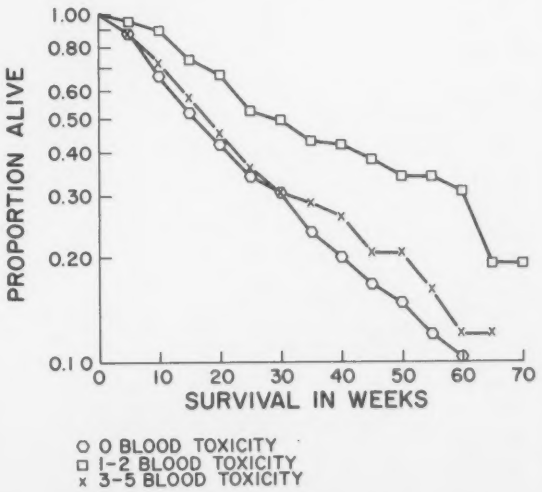

FIGURE 5.—EST-0870 and EST-4273. Relationship between hematologic toxicity and survival among patients with advanced colorectal carcinomas treated with CCNU and methyl-CCNU.

METHYL-CCNU AS A SINGLE AGENT

Methyl-CCNU showed a response rate of 8% when given as a solitary chemotherapeutic agent for carcinoma of the stomach. In carcinoma of the colon and rectum a 17% response rate was seen and in carcinoma of the pancreas 9% of the patients responded. Activity was also demonstrated in biliary tract carcinoma. In all studies major hematologic toxicity occurred in approximately 20% of all the patients treated, but < 10% of the patients had either severe bleeding or vomiting (table 5).

In pancreatic cancer, the response rate to the combination of streptozotocin plus 5-FU seemed to be somewhat higher than that of methyl-CCNU alone; streptozotocin alone was not evaluated. The median survival in both groups, however, was the same (15 weeks).

RESULTS IN GASTRIC CANCER

Evaluation of Methyl-CCNU Alone or in Combination With 5-FU

Patients with advanced incurable gastric carcinoma were treated with methyl-CCNU or with the combination of methyl-CCNU and 5-FU in a program designed by Dr. Moertel (Mayo Clinic, Rochester, Minn) in 1972. The combination therapy resulted in an increase in the frequency of severe hematologic toxicity, but in other parameters the toxic effects were essentially unchanged from those seen when methyl-CCNU was given alone. Of the patients treated with methyl-CCNU and 5-FU, 47% had severe hematologic toxicity and 13% had severe vomiting (fig 10). In contrast, only 22% of the patients treated with methyl-CCNU alone showed severe hematologic toxicity, but vomiting and bleeding each occurred in 8% (table 6).

Although the methyl-CCNU and 5-FU toxicity was much increased compared to that of methyl-CCNU alone, the response rate was markedly im-

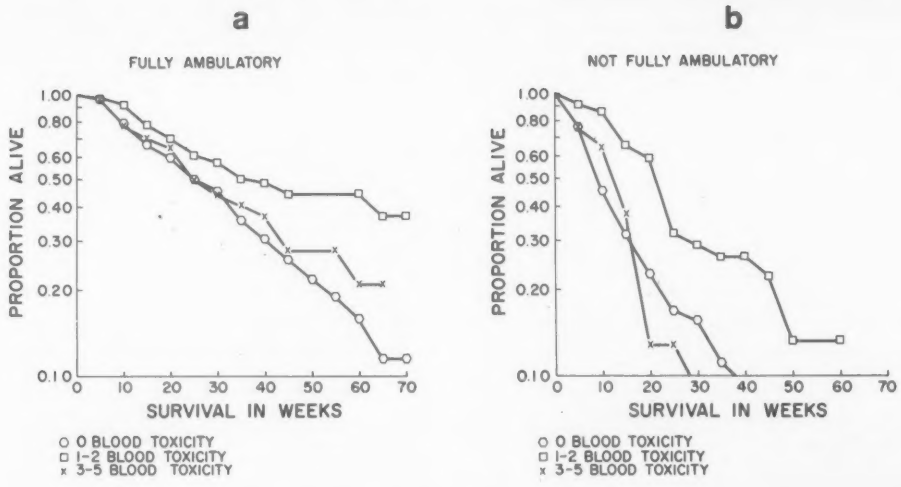

FIGURE 6.—EST-0870 and EST-4273. Interaction between nitrosourea-induced drug toxicity and physical status and their effect on survival of patients with advanced colorectal carcinoma. The best survival occurred in patients who had mild to moderate hematologic toxicity.

TABLE 5.—Results of single agent therapy with methyl-CCNU for various GI adenocarcinomas: preliminary data for biliary tract tumors suggest drug activity

Protocol (EST No.)	Organ site	No. of responses/ No. of patients evaluable	Median survival (wks)	Major toxic effects (≥ severe)		
				Hematologic	Bleeding	Vomiting
4273	Colon + rectum	9/54 (17%)	31	21%		9%
1272	Stomach	3/37 (8%)	10	22%	8%	8%
2272	Pancreas	3/34 (9%)	12	18%		
2273	Gallbladder + bile duct		—	33%		

TABLE 6.—Synergistic response rates when 5-FU is added to methyl-CCNU in the treatment of advanced gastric adenocarcinoma; toxicity was also increased (EST No. 1272)

Drug, mg/m² (7-wk cycle)	No. of responses/ No. of patients evaluable	Median survival (wks)	Severe toxicity		
			Hematologic	Bleeding	Vomiting
Methyl-CCNU, 200	3/37 (8%)	10	22%	8%	8%
Methyl-CCNU, 175 + 5-FU, 300, Days 1–5	12/30 (40%)	22	47%		13%

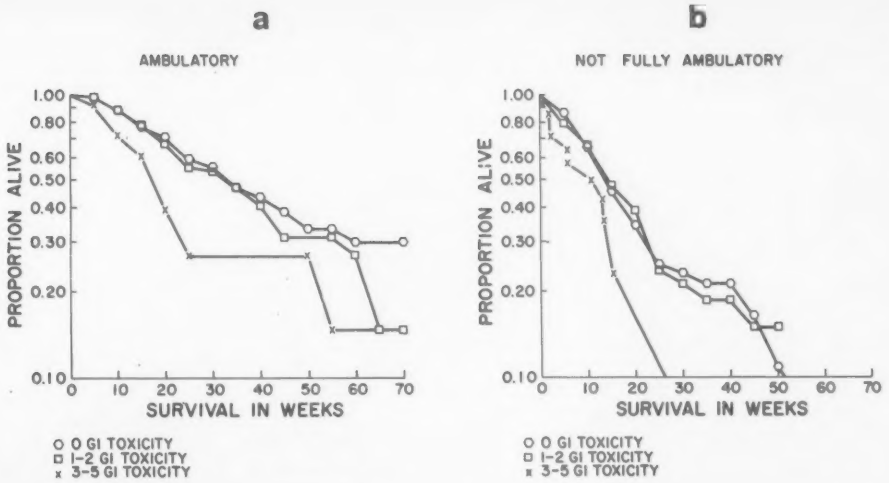

FIGURE 7.—EST-0870 and EST-4273. Upper GI toxicity (eg, vomiting) among patients with advanced colorectal carcinoma treated with methyl-CCNU and CCNU. Whether patients were ambulatory or not, there was no benefit obtained in association with any degree of toxicity.

proved. Whereas only three of 37 patients treated with methyl-CCNU alone showed objective PRs (a median survival of 10 weeks), 40% of the patients treated with the combination of methyl-CCNU and 5-FU had objective responses. The mean survival for patients treated with the combination was increased to 22 weeks. It is interesting to note that among these patients, five of five who had had partial gastrectomies for adenocarcinoma and now had recurrent metastatic disease showed objective responses. Four of eight patients who were treated by gastroenterostomy without resection and two of seven treated by total gastrectomy showed objective responses. Only two of 11 patients who had neither bypass nor resection showed objective response.

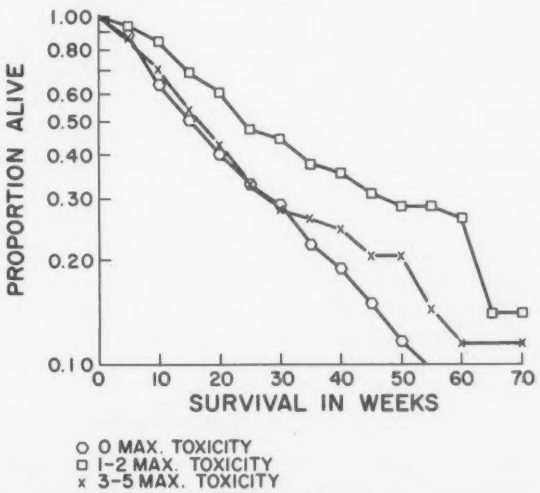

FIGURE 8.—EST-0870 and EST-4273. Effect of total drug toxicity on survival in patients with advanced colorectal adenocarcinoma who were treated with methyl-CCNU or CCNU. Although a little toxicity appears necessary for prolonged survival, increased toxicity does not benefit the patient.

The response rate was little different whether the tumor remained in situ or had been removed, but, again, there was a marked correlation with physical status. Six of 11 ambulatory patients showed objective PR with the combination of methyl-CCNU and 5-FU, whereas only two of 11 nonambulatory patients responded. Methyl-CCNU alone had given two responses among 18 ambulatory patients and no responders in the nonambulatory group.

Cyclophosphamide Induction

Administration of cyclophosphamide 3 weeks before treatment with methyl-CCNU or with 5-FU and methyl-CCNU (table 7) resulted in a marked increase in the incidence and severity of hematologic toxicity and in vomiting. The response rates among these patients were reduced; cyclophosphamide appeared to detract from the effect of chemotherapy with methyl-CCNU plus 5-FU. Objective remissions occurred in 19% of the patients while 45% had severe hematologic toxicity and 13% had intractable vomiting. Among patients who received methyl-CCNU without 5-FU, the addition of cyclophosphamide altered the response rate only slightly, but

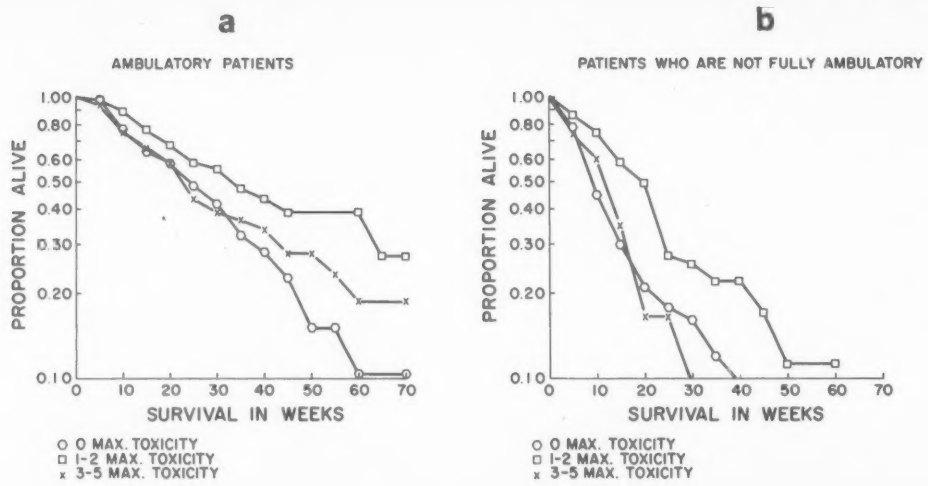

FIGURE 9.—EST-0870 and EST-4273. Effect of maximum drug toxicity on survival of fully ambulatory patients and of those who were not fully ambulatory treated with CCNU and methyl-CCNU for advanced colorectal adenocarcinoma.

increased the incidence of severe hematologic toxicity.

Adjuvant Studies With Methyl-CCNU and 5-FU

As a result of the apparent increased sensitivity of gastric cancer to the combination of 5-FU and methyl-CCNU, adjuvant studies in patients who have had complete resection of their gastric carcinoma have been initiated by the ECOG in cooperation with the GITSG. GITSG is also using nitrosoureas with 5-FU in patients who appear to have only local residual disease. These programs have been in progress for > 1 year and it is too early to determine their efficacy. Nevertheless, toxicity data (particularly, toxicity requiring dose reduction) are now available. In the patients receiving adjuvant chemotherapy with BCNU plus 5-FU (table 8), 43% have required dose reduction because of hematologic toxicity. Methyl-CCNU, which was substituted for BCNU at a 15% lower dose than that used in the treatment of advanced gastric cancer, was better tolerated with a dose reduction required in only 33% of the patients. Among patients with local residual disease (ie, positive suture lines, lymph nodes left behind, and locally unresectable cancer with no metastases), the frequency of dose reduction in patients treated with BCNU and 5-FU was 40%, whereas among those treated with methyl-CCNU plus 5-FU only 8% have required dose reduction thus far. Antecedent radiotherapy (≤ 5000 rads to the upper abdomen) altered the toxicity of this combination of agents only slightly. Although dose reduction became necessary in a number of patients, the toxicity that precipitated these changes often was not apparent after the first treatment course. Dose reduction was sometimes instituted after the 2nd, 3rd, or 4th courses of adjuvant chemotherapy. The necessity for continued followup of blood counts continued to be ever-present throughout the entire program of adjuvant therapy.

Other Trials of 5-FU and Methyl-CCNU

Subsequent studies in patients with advanced gastric cancer using 5-FU plus methyl-CCNU were initiated with a downward adjustment in the dose of methyl-CCNU and a compensatory increase in the dose of 5-FU (table 9). Even though it is too early to determine the response rate in these newer programs, their desired objective has been attained; toxicity has been nearly halved. Only 24% of the patients receiving 150 mg of methyl-CCNU every 10 weeks accompanied by 325 mg of 5-FU iv on Days 1–5 and then increased to 375 mg/m² on Days 36–40 have had severe hematologic toxicity with severe vomiting in only 8%. Previously, severe hematologic toxicity occurred in 47% of the patients treated and severe vomiting in 13%.

Examinations of the therapy results with this combination of drugs are also under way in pancreatic carcinoma and in biliary and hepatic tumors. Among the latter, 5-FU had been given orally. The response rates have been low in hepatocellular neoplasms but appear more promising in patients with biliary carcinoma. Major hematologic toxic effects

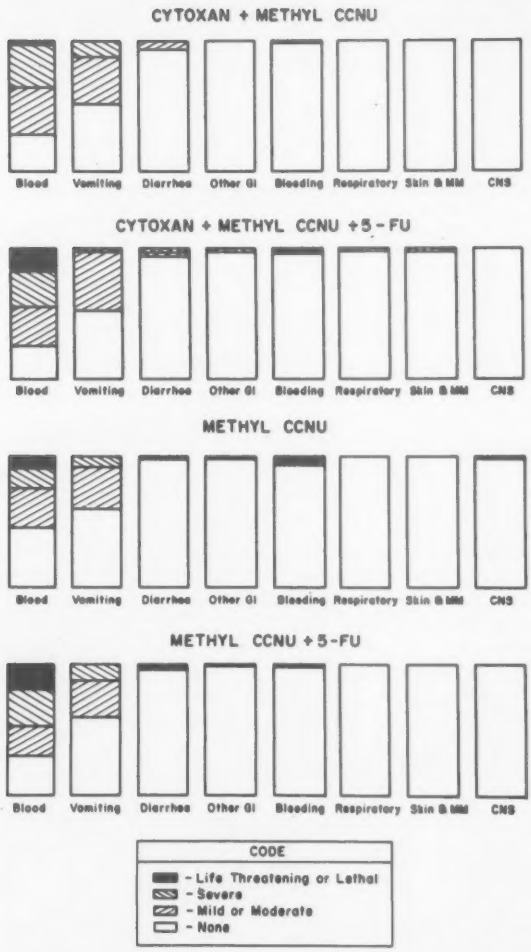

FIGURE 10.—Comparative toxicities of methyl-CCNU, alone or in combination with 5-FU, and the effect of cyclophosphamide (Cytoxan) induction. Each bar represents an arithmetic scale of 100% of patient data available for that toxicity parameter.

have been noted in 21% of the patients; vomiting has been severe in 12%. At present, patients with hepatocellular tumors are now given 5-FU iv.

Methyl-CCNU and Other Agents

A report from Denver in 1973 (3) suggested that testolactone and spironolactone might greatly prolong the lives of patients with pancreatic and gastric carcinoma. To test this theory, these drugs were administered to patients receiving 5-FU plus methyl-CCNU (table 7). Because survival seemed to be the parameter most affected, the evaluation was initiated as a survival study in patients with nonmeasurable disease. Since this is a survival study, another year will be needed to detect whether or not there has been a significant change introduced by spironolactone and testolactone. There has been no change in the toxic effects.

Methyl-CCNU and 5-FU have been added to streptozotocin, a second non-cross-reacting nitrosourea, in patients with pancreatic cancer (4). This study has only recently been initiated.

6-Thioguanine was also included in our early trial of CCNU and streptozotocin in colorectal carcinoma. A few long-term objective responders to thiogua-

TABLE 7.—Early results of trials by ECOG with methyl-CCNU in combination with other chemotherapeutic agents

Protocol (EST No.)	Organ site	Methyl-CCNU: dose (mg/m²) and schedule	Other agents: dose (mg/m²) and schedule	Response rate	Severe toxic effects		Comment
					Hematologic	Other	
1272	Stomach	175, q7wks, begin Day 22	Cyclophosphamide induction, 1200, Day 1	7%	37%		Cyclophosphamide induction detracted from methyl-CCNU + 5-FU
			Cyclophosphamide induction + 5-FU, 300, Days 22–26	19%	45%	13% (vomiting)	
4274	Stomach	150, q10wks	5-FU, alternating 325–375 Testolactone, 50 mg, tid	Nonmeasurable	33%	11% (vomiting) 11% (bleeding)	
2275	Pancreas	150, q8wks	5-FU, alternating 350–400 Streptozotocin, 400, Days 1–5	Nonmeasurable			
1275	Colorectal	150, q8wks	β-TGdR, 60, Days 1–5, 29–33				

TABLE 8.—Nitrosourea-5-FU combination chemotherapy in locally confined gastric cancer; adjuvant trials after complete surgical excision and therapy of local residual and locally unresectable tumors that have not metastasized beyond regional lymph nodes

Protocol (EST No.)	Purpose	Drug	Dose (mg/m²) and schedule	Toxicity requiring dose reduction—		
				Hematologic	GI	Stomatitis
8174	Adjuvant	BCNU 5-FU	40 × 5, q10wks 400 × 5, q5wks	43%		
		Methyl-CCNU 5-FU	150, q10wks 325 × 5 then 375 × 5	33%		
8274	Local residual	BCNU 5-FU	40 × 5, q10wks 400 × 5, q5wks	40%	40%	20%
		Methyl-CCNU 5-FU	150, q10wks 325 × 5 then 375 × 5	8%	8%	
	Radiotherapy	BCNU 5-FU	40 × 5, q5wks 400 × 5, q5wks	33% 21%	33%	
		Methyl-CCNU 5-FU	150, q10wks 325 × 5 then 375 × 5			7%

TABLE 9.—Dose levels and toxic effects in ECOG upper GI organ site trials of methyl-CCNU and 5-FU

Protocol (EST No.)	Organ site	Dose (mg/m²)/schedule		Major toxic effects (≥ severe)	
		Methyl-CCNU	5-FU	Hematologic	Vomiting
1272	Stomach	175/q7wks	300/iv × 5 q7wks	47%	13%
3274 4274 3275	Stomach	150/q10wks	325/iv, Days 1–5 375/Days 36–40	24%	8%
2275	Pancreas	150/q10wks	350/iv, Days 1–5 400/Days 36–40		
2273	Liver, gallbladder, bile duct	150/q10wks	500/orally, Days 1–5, 36–40	21%	12%

nines combined with the apparent evidence of a lack of cross resistance between its equally active derivative, β-2′-deoxythioguanosine (β-TGdR), and nitrosoureas, have resulted in a trial of methyl-CCNU and β-TGdR in advanced, previously treated, and drug-resistant colorectal carcinomas. This program has also been recently activated. However, when utilized as a single agent in previously treated colorectal cancer, β-TGdR had yielded some objective PRs. A possible increase in the duration of patient survival was also noted.

DISCUSSION

When used as single agents, it would appear that some nitrosoureas have the same activity as 5-FU in gastric and colorectal carcinoma. Objective responses occurred in eight of 50 patients treated with methyl-CCNU and in six of 20 treated with CCNU when no prior chemotherapy had been given. A lack of activity in 17 patients treated with streptozotocin would suggest that this is an inferior drug for colorectal carcinoma. But, the responses to streptozotocin in patients who had had prior chemotherapy, even though not reflected in terms of overall survival, should not be overlooked.

Present information would indicate that response is most likely to occur after the nitrosourea has been administered at a level that will result in a modest, but tolerable, toxicity. Increasing the dose of the drug increased the extent and frequency of toxicity without altering the response rate. The patients with more severe toxic effects responded as badly as those who may have been receiving an insufficient drug dose. This phenomenon was unrelated to physical status; it occurred in both ambulatory and nonambulatory patients. There was no relationship between the degree of nausea and vomiting and the response rate. Nausea and vomiting are expected central nervous system actions of the drugs and, perhaps, should not be considered "toxicity."

Methyl-CCNU alone had little activity in gastric carcinoma. Its effect was only slightly different from that to be expected using 5-FU. The combination of methyl-CCNU and 5-FU resulted in a dramatic increase in both the response rate and in the median survival time. This dramatic increase was associated with a significant increase in the frequency of severe or life-threatening hematologic toxicity, but there were no patients whose deaths could be attributed in any way to chemotherapy.

Since methyl-CCNU was given orally, it was interesting to evaluate how the various surgical derangements in the GI tract would affect the tumor response. Patients who had had a gastroenterostomy, either as part of a resection or as a bypass, tolerated the nitrosoureas quite well. There were also responders among patients who had total gastrectomies. Fewer responses were recorded in patients in whom the tumor remained in situ and in whom no bypass had been done.

The synergistic effect of these chemotherapeutic agents could have been predicted in the animal studies by Schabel's group, who had also shown a lack of cross resistance with alkylating agents (5). Attempts to induce a tumor response with cyclophosphamide and then maintain it with nitrosoureas failed in human gastric cancer. Indeed, cyclophosphamide appeared to detract from the nitrosourea-5-FU reactivity.

Schabel had also suggested the use of nitrosoureas as adjuvant chemotherapy agents based on studies with B16 melanoma and colon tumor 26 in BALB/c mice.[7] In the colon tumor, chemotherapy was beneficial when administered after surgery but not when given before. The application of this principle to human cancer has begun with drug dosage schedules that have quite acceptable levels of toxicity. Cumulative toxicity has been suggested by the appearance of hematologic changes requiring dose reduction after two, three, or four complete courses of treatment. Once a patient has tolerated four courses, future dose reduction may be less common. Prior upper abdominal high-dose radiation therapy does not seem to increase the toxicity even though as many as 5000 rads have been administered.

Activity of methyl-CCNU and 5-FU has also been demonstrated in biliary tract carcinoma and is being studied in hepatocellular and pancreatic carcinoma. Among hepatoma patients with cirrhosis, the response rate to chemotherapy is reduced.

Evaluation of the interaction of 5-FU and methyl-CCNU with other drug combinations is under way, but results are too preliminary to estimate activity.

LIST OF COMPOUNDS

BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-

Bleomycin: NSC-125066; [2,4′-bithiazole]-4-carboxylic acid, 2′-(2-aminoethyl)-, monohydrate

CCNU: NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate

DTIC: NSC-45388; CAS reg. No. 4342-03-4; imidazole-4-carboxamide, 5-(3,3-dimethyl-1-triazeno)-

5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8

Hydroxyurea: NSC-32065; CAS reg. No. 127-07-1

Inosine dialdehyde: NSC-118994; diglycolaldehyde, α-(hydroxymethyl)-α′-(6-hydroxy-9*H*-purin-9-yl)-

[7] *See* footnote 6.

Methyl-CCNU: NSC-95441; CAS reg. No. 33073–59–5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-

Procarbazine: NSC-77213; CAS reg. No. 491–88–3; *p*-toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, monohydrochloride

Spironolactone: NSC-150399; CAS reg. No. 52–01–7; 17α-pregn-4-ene-21-carboxylic acid, 17-hydroxy-7α-mercapto-3-oxo-, γ-lactone, acetate

Streptozotocin: NSC-85998

Testolactone: NSC-23759; CAS reg. No. 968–93–4; 1-phenanthrenepropionic acid, 1,2,3,4,4*a*,4*b*,7,9,10,10*a*-decahydro-2-hydroxy-2,4*b*-dimethyl-7-oxo, δ-lactone

β-TGdR: NSC-71261; 9*H*-purine-6-thiol, 2-amino-9-(2-deoxy-β-D-erythro-pentofuranosyl)-, monohydrate

6-Thioguanine: NSC-752; CAS reg. No. 1832–72–0; purine-6-thiol, 2-amino-

Vincristine: NSC-67574; CAS reg. No. 2068–78–2; leurocristine, sulfate (1:1)

VP-16-213: NSC-141540; 4′-demethylepipodophyllotoxin 9-(4,6-*O*-ethylidene-β-D-glucopyranoside)

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Southeastern Cancer Study Group Trials With Nitrosoureas in Hodgkin's Disease

John R. Durant,[1,*] Alfred Bartolucci,[1] Richard A. Gams,[1] Ronald F. Dorfman,[2] and Enrique Velez-Garcia[3]

SUMMARY

A decade of studies with the nitrosoureas in the Southeastern Cancer Study Group has shown that they are active agents for the treatment of Hodgkin's disease and that they may be combined with other chemotherapeutic agents in regimens which have acceptable toxicity to produce excellent response rates. Furthermore, six monthly cycles of treatment with a combination of either BCNU, vinblastine, cyclophosphamide, procarbazine, and prednisone or mechlorethamine, vincristine, prednisone, and procarbazine (MOPP), following the achievement of a clinical CR, produce significantly superior durations of remission and survival in previously untreated patients. Our studies with BCNU given orally have indicated that it is not a clinically useful drug. Finally, studies with methyl-CCNU given orally have indicated no particular place for this agent in the treatment of Hodgkin's disease when compared to CCNU and BCNU.

[Cancer Treat Rep 60:781–787, 1976]

The Southeastern Cancer Study Group (SESG) first became interested in the nitrosoureas nearly a decade ago. This interest resulted from studies of these agents showing a broad spectrum of activity in many animal systems as well as early clinical reports of activity in man. This report summarizes seven studies with the nitrosoureas in Hodgkin's disease performed by the SESG beginning in 1966.

Our first study used BCNU given alone iv and has been reported in detail elsewhere (1). A total of 66 evaluable patients with chemotherapeutically refractory stage III-B and IV Hodgkin's disease were treated with BCNU given iv at doses of 100 mg/m^2 on 2 or 3 consecutive days every 4 weeks. Thirty of 66 patients had a useful response with four patients (7%) achieving CR. Nausea and vomiting occurred in approximately one fourth of the patients. Hematologic toxicity (primarily delayed thrombocytopenia and granulocytopenia) in these patients followed the pattern reported by many others. Slightly more than half of the patients had significant reductions in the platelet count, and in 68% of these it fell to 50,000 cells/mm^3 or less.

The combination of these therapeutic effects and the difficulty associated with iv infusion prompted us to begin a study in 1968 of BCNU given orally in a variety of doses and schedules. This study has been previously reported (2). BCNU, given by this route, produced dose-limiting gastrointestinal toxicity with very little effect on the bone marrow. Furthermore, in 26 patients no CRs and only five PRs were obtained. We concluded that BCNU given orally was impractical and abandoned further studies with the oral form of this agent.

Subsequently, beginning in 1972, we attempted a phase I and II study using methyl-CCNU in doses between 50 and 200 mg/m^2 in patients with far-advanced, chemotherapeutically refractory Hodgkin's disease. Many of these patients had previously received BCNU given iv. This study, which will be reported in detail elsewhere,[4] indicated that the drug was reasonably well tolerated, but that there were few useful responses (four PRs among 29 patients) in this group of patients. Because of the

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[4]Tornyos K. Unpublished SESG data.

apparent inferiority of methyl-CCNU to CCNU as reported by Holland,[5] we have discontinued using this agent in the treatment of Hodgkin's disease.

Our more important studies have had to do with the development of combination regimens using iv BCNU. These studies were started because of evidence in a variety of animal tumor systems that the nitrosoureas were synergistic with cyclophosphamide (3).[6] Thus, we were motivated to develop BCNU and cyclophosphamide combinations which could serve as alternatives to the combination of mechlorethamine, vincristine, prednisone, and procarbazine (MOPP) in the treatment of Hodgkin's disease. These studies have also been reported in detail elsewhere (4). We began by combining BCNU and vinblastine or procarbazine in stages III-B and IV Hodgkin's disease patients who were refractory to other forms of chemotherapy. The data obtained from these studies indicated that it was possible to give as much as 200 mg/m² of BCNU in combination with vinblastine (5 mg/m² given iv every 4 weeks), and as much as 150 mg/m² of BCNU every 4 weeks in combination with procarbazine (100 mg/m²/day × 10 days every 4 weeks). These data were derived from six patients; one had stage IV-B Hodgkin's disease involving the marrow with almost continuous activity for 8 years. This patient had a CR with BCNU and procarbazine and now has had an unmaintained CR for > 5½ years. Thereafter, we tested the three four-drug combinations shown in table 1. The data indicated that the regimens administered produced significant but tolerable hematologic and gastrointestinal toxicity and comparable response rates in 61 patients with Hodgkin's disease. Most of these patients had had extensive prior therapy including radiation and multiple-drug therapy. The regimen in which vincristine was substituted for cyclophosphamide produced a neuromyalgic syndrome which was often prolonged and severe. Since it did not produce a startlingly superior response rate and did not contain cyclophosphamide, further testing of this regimen was discontinued after only eight patients were treated. Although this was a feasibility study with treatment given mostly to patients who had had major prior chemotherapeutic exposure, it is interesting to note that five of 21 patients who achieved CR have required no therapy for > 5 years.

[5]Holland JF. Studies of the nitrosoureas in Hodgkin's disease: studies of Acute Leukemia Group B. Presented at the Proceedings of the 7th New Drug Seminar on Nitrosoureas, Dec 15–16, 1975.

[6]Schabel FM, Jr. Personal communication.

TABLE 1.—Nitrosoureas in Hodgkin's disease: 4-drug BCNU and cyclophosphamide combinations in stages III and IV refractory Hodgkin's disease (1969–1971)*

Drugs (route)	Dose (mg/m²) and schedule	No. of evaluable patients	Response				No. of patients with toxicity				
			Complete†	Partial	None	%	Hemoglobin	Granulocytes	Platelets	Gastrointestinal	Central nervous system
BCNU (iv) Cyclophosphamide (iv) Vincristine (iv) Procarbazine (oral)	100, q28d 600, q28d 1, q14d 100, × 10 days	31	9	14	8	74 (23/31)	2	16	9	12	13
BCNU (iv) Vincristine (iv) Vinblastine (iv) Procarbazine (oral)	100, q28d 1, q14d 5, q28d 100, × 10 days	8	3	2	3	62 (5/8)	1	2	3	5	1
BCNU (iv) Cyclophosphamide (iv) Vinblastine (iv) Procarbazine (oral)	100, q28d 600, q28d 5, q28d 100, × 10 days	22	9	8	5	77 (17/22)	4	8	1	10	2

*From ref 4.

†Five patients continue in their original CR 5 yrs from start of study.

In our most recent study, we selected the regimen of BCNU, cyclophosphamide, vinblastine, and procarbazine because: (*a*) it was more different from MOPP than the regimen containing vincristine substituted for vinblastine; (*b*) it required only one visit a month; and (*c*) there was a very low incidence of peripheral neuropathy. To these drugs we added prednisone at a dose of 60 mg/m^2/day for 10 days and designed a study in cooperation with the Eastern Cooperative Oncology Group (ECOG). It was agreed that the ECOG would compare the response rates to those of MOPP whereas the SESG would attempt to induce remissions with six monthly cycles of a combination of BCNU, vinblastine, cyclophosphamide, procarbazine, and prednisone (BVCPP), with patients stratified according to amount of prior therapy, and then the value of further chemotherapy in those achieving a CR would be studied. These responding patients were randomized either to continued induction therapy for six more cycles, to six monthly cycles of MOPP, or to no therapy. This study was begun in July 1971 and the accession of patients was completed in December 1975. Incomplete results have been previously presented in abstract form (5,6). The results are derived from an analysis performed in October 1975. Two hundred and seventy-one of 327 eligible patients were evaluable and 56 were unevaluable. In 19 of the unevaluable patients (6%) there was a major protocol violation. In an additional 37 patients (11%) the data were inadequate for evaluation; in 28 of these patients this was because the patient refused additional therapy, usually while in CR. These patients were considered unevaluable for this study because the goal was to determine the effect of continued therapy on patients who had achieved CR following six cycles of induction therapy. The majority of these unevaluable patients came from three institutions. In two of these institutions a significant number of patients did not speak English and left the area when they were feeling better. In the third institution many patients were required to travel for hundreds of miles to receive treatment and were unwilling to continue because of transportation problems. Despite these problems, however, 82% of the eligible patients who were entered in the study were able to complete the induction regimen.

RESULTS

The response rates for the induction phase of the protocol are seen in table 2. These are comparable to results with MOPP with a CR rate of about 65% in previously untreated patients. Figure 1 shows the response rate according to the type of prior therapy in 126 patients who had received major prior therapy according to our definition (7). It can be seen that prior exposure to many chemotherapeutic agents or resistance to one of the agents in the regimen adversely affected the CR rate ($P < 0.01$).

Table 3 shows the significant toxicities for this study. Five percent of the previously untreated patients had life-threatening depression of the bone marrow, and 8% of those with major prior therapy had a similar complication. It can be seen from the table that the granulocyte toxicity was both more frequent and severe than the platelet toxicity. Slightly less than half of the patients had significant nausea and vomiting. Neurotoxicity was negligible and occurred in only 3% of all the patients in this study.

Table 4 shows the deaths during and after the study. As in our previous study of MOPP versus mechlorethamine (7) approximately 10% of the patients died during induction therapy. One half of these were due to progressive and unresponsive disease. As with other cancers (8), approximately 25% of the patients who died in this study died of unrelated diseases.

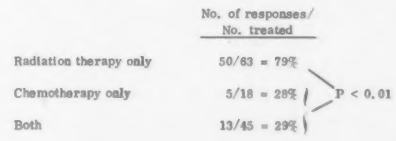

FIGURE 1.—Therapy for Hodgkin's disease with BVCPP: CR by type of major prior therapy.

TABLE 2.—BVCPP in Hodgkin's disease: response rates according to major prior therapy

	No. of—			Total % responding
	Patients	CRs	Good PRs	
Minimal or no prior therapy	145	102 (70%)	14 (10%)	80
Major prior therapy	126	68 (54%)	12 (10%)	64

TABLE 3.—Major toxicities of BVCPP according to amount of prior therapy*

		No. with toxicity/No. treated		
	Degree of toxicity	Granulocytes	Platelets	Gastro-intestinal
Minimal or no prior therapy	Life threatening	7/145 (5%)	4/145 (3%)	
	Other	66/145 (46%)	43/145 (30%)	69/145 (48%)
	Total %	51%	33%	48%
Major prior therapy	Life threatening	10/126 (8%)	1/126 (1%)	
	Other	57/126 (45%)	54/126 (36%)	58/126 (46%)
	Total %	53%	37%	46%

*There were 3 fatal pulmonary complications: 2 drug related (biopsy) and 1 viral pneumonia.

TABLE 4.—Deaths in 329 patients receiving BVCPP

	No. of deaths	
Cause	During induction	Subsequent to induction
Disease	16 (50%)	13 (52%)
Toxicity	7 (31%)	1 (4%)
Unrelated	6 (19%)	8 (32%)
Unknown	3 (10%)	3 (12%)
Totals	32/329 (10%)	25/329 (8%)

Total deaths 57/329 (17%)
Total unrelated deaths 14/57 (25%)

The dosage data are shown in table 5. In our studies we use a dose/time relationship (9). The percent of the targeted dose administered over the entire course of therapy is multiplied by the targeted time for completion divided by the actual time necessary to administer the drugs. It can be seen that previously untreated patients received between 80% and 90% of the anticipated dose, and that those with major prior therapy received approximately 10% less. During the maintenance phase of therapy patients without major prior therapy were able to tolerate no more drug than patients who had previously received major prior therapy.

An analysis of factors influencing the response rates, ie, age, sex, stage, histologic type, institution, splenectomy, and initial lymphocyte count, is being conducted but as yet is incomplete. The most important data available from this study are, however, not the response rates or toxicity data, but rather the differences in remission duration and survival which are apparent in patients without major prior therapy who receive an additional 6 months of treatment after achieving CR. Figure 2 shows that of 34 patients without major prior therapy who were randomized to six additional cycles of therapy, only four have relapsed. Their chances of remaining in remission for 36 months after completion of therapy are approximately 87%. Of 27 patients who were randomized to receive no additional therapy, nine have relapsed, and the chances of their remaining in remission at 36 months are only 67%. This difference is significant ($P = 0.023$). Furthermore, figure 3 shows that the superior duration of CR in the maintained patients is reflected in superior survival. A patient without major prior treatment who achieved a CR and was treated for an additional 6 months had a 97% chance of surviving at 48 months, whereas similar patients without additional therapy had only a 67% chance of surviving during the same period. All the deaths in this group were due to Hodgkin's disease, and this difference is also significant ($P = 0.036$).

The favorable effect of continuing therapy which was seen in patients without major prior therapy is not seen in those who developed CR on BVCPP after relapsing following major prior therapy (radiation, chemotherapy, or a combination). The median duration for CR for this group is 22 months. Further, remission duration and survival of those achieving a CR and who had received only major prior radiation therapy are not different from those who had received other forms of major prior therapy.

DISCUSSION

MOPP therapy is the therapeutic regimen of choice for stage III-B and IV Hodgkin's disease (10). Our study and others reported at this symposium indicate that combination therapies including either BCNU or CCNU provide an effective alternative to MOPP chemotherapy. The frequency of CR is approximately equivalent, and although toxic effects of all kinds appear to be fewer, hematologic toxicity is still frequent. There is a sparing of the platelets as might be expected by the substitution of cyclophosphamide for mechlorethamine and the inclusion

TABLE 5.—Dose administered over time of individual drugs in BVCPP according to amount of prior therapy

	% of targeted dose given				
	BCNU	Vinblastine	Cyclophosphamide	Procarbazine	Prednisone
Induction therapy					
Minimal or no prior therapy (125 patients)	86.5%	91.2%	85.0%	82.8%	92.3%
Major prior therapy (102 patients)	77.4%	79.3%	75.2%	72.3%	88.0%
Maintenance therapy					
Minimal or no prior therapy (125 patients)	67.4%	70.6%	66.2%	62.4%	102.6%
Major prior therapy (102 patients)	71.9%	82.4%	67.5%	71.3%	94.4%

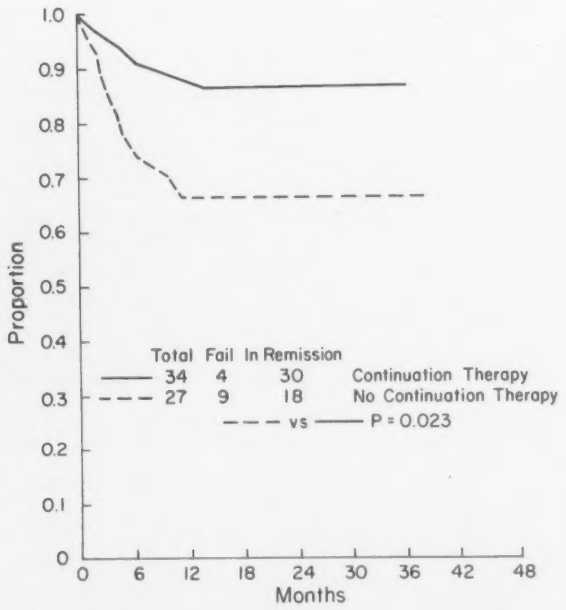

FIGURE 2.—Duration of CRs from end of therapy in previously untreated patients according to whether or not continuation therapy was given.

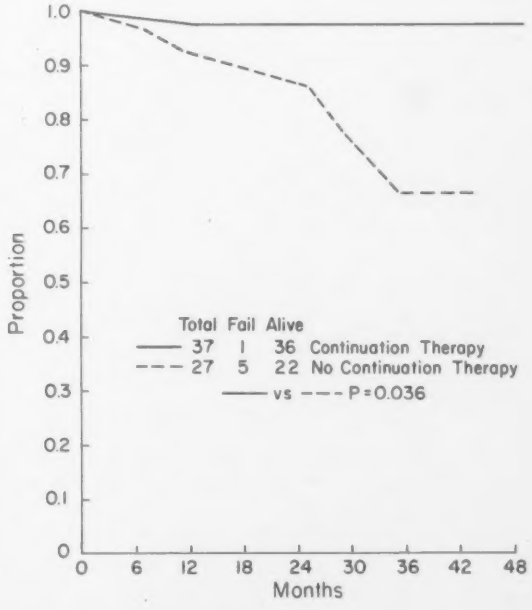

FIGURE 3.—Survival of previously untreated patients with CR from start of study according to whether or not continuation therapy was given.

of vinblastine. These agents would be expected to limit the doses of other granulocytopenic agents without themselves producing significant thrombocytopenia. The very low incidence of neurologic toxicity is a clear therapeutic advantage for the nitrosourea regimens. There are, however, no data to suggest that BCNU and cyclophosphamide are synergistic in this combination given in this particular dose and schedule.

Of great interest is the controversy regarding the value of continued therapy in Hodgkin's disease. Various studies done by the clinical staff at the NCI have indicated no advantage to maintaining MOPP-induced CRs with either additional MOPP or a variety of single agents (11,12). The Southwest Oncology Group (SWOG) has previously reported a superior duration of CR and unmaintained CR for patients receiving MOPP every other month for 18

months following CR with MOPP (13). A followup on this study has shown that after 7 years there is no longer an advantage for the maintained patients.[7] The data presented in our study are in marked contrast to those of the SWOG study because previously untreated patients who receive additional therapy have both a significantly longer duration of unmaintained CR and superior survival. Although the curves for remission duration and survival may subsequently come together, the considerable advantage in intermediate-length survivals ($\leq$ 4 years) for those receiving additional treatment strongly suggests that six cycles of additional therapy following CR is of value in previously untreated patients.

It may be argued that the patients reported in this study were not rigorously restaged, and that the induction regimen described produced some pseudo-CRs which were rendered complete by additional therapy. Although this possibility exists, the practicalities of rigorously restaging patients will ordinarily not permit its being done in a systematic fashion. Furthermore, modern statistical techniques should allow an analysis of pretreatment characteristics in the various groups of patients in this and other studies, thus permitting an analysis of the characteristics of those patients who achieved continuous CR without maintenance therapy as opposed to those who did not. It is hoped in this way to study further the question of maintenance therapy. On the other hand, we believe that we have satisfactorily demonstrated that relapse following major prior therapy is an indication for only six cycles of therapy, especially since a combination of radiation therapy and chemotherapy appears synergistically carcinogenic in patients with Hodgkin's disease (14,15). It should be especially noted that although the CR rate in patients relapsing from radiation therapy is equivalent to or superior to that of previously untreated patients, the subsequent performance of these patients in terms of the duration of their CR and survival is definitely inferior to that of those with no prior therapy. This may suggest that a disease which relapses is more difficult to completely eradicate even though it is quite responsive to various therapies. There may be other factors which will emerge from an analysis of the pretreatment status which may indicate other ways of predicting which patients should receive > 6 months of induction therapy.

Finally, it should be noted that although the findings reported here are controversial and are contrary to the conclusions reached by the NCI and to some extent by the SWOG, our results with BCNU combinations and continuation therapy after CR in Hodgkin's disease are consistent with the findings of the Acute Leukemia Group B in which maintenance of their CCNU and vinblastine combination-induced CR produces superior long-term results (16).

[7]Coltman CA, Jr. Personal communication.

LIST OF COMPOUNDS

BCNU: NSC-409962; CAS reg. No. 154–93–8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-

BVCPP = BCNU, vinblastine, cyclophosphamide, procarbazine, and prednisone

CCNU: NSC-79037; CAS reg. No. 13010–47–4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-

Cyclophosphamide: NSC-26271; CAS reg. No. 6055–19–2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate

Mechlorethamine: NSC-762; CAS reg. No. 55–86–7; diethylamine, 2,2′-dichloro-*N*-methyl-, hydrochloride

Methyl-CCNU: NSC-95441; CAS reg. No. 33073–59–5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-

MOPP = mechlorethamine, vincristine (Oncovin), prednisone, and procarbazine

Prednisone: NSC-10023; CAS reg. No. 53–03–2; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-

Procarbazine: NSC-77213; CAS reg. No. 491–88–3; *p*-toluamide, *N*-isopropyl-α-(2-methylhydrazino)-, monohydrochloride

Vinblastine: NSC-49842; CAS reg. No. 6449–03–2; vincaleukoblastine, sulfate (1:1), monohydrate

Vincristine: NSC-67574; CAS reg. No. 2068–78–2; leurocristine, sulfate (1:1)

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Nitrosoureas in Multiple Myeloma[1]

Sydney E. Salmon[2,3,*]

SUMMARY

A number of cooperative-group and single-institution studies have shown that BCNU used in combination with prednisone alone or with melphalan, cyclophosphamide, and prednisone is useful for remission induction in patients with previously untreated multiple myeloma. In this setting, results with BCNU (and probably CCNU) are as good as (but not superior to) the results obtained with other simple combinations such as melphalan plus prednisone with regard to the frequency of remission induction, duration of remission, and survival. BCNU plus prednisone also appears to be equal to melphalan plus prednisone for remission-maintenance therapy, although it is still unclear whether maintenance therapy is superior to discontinuation of therapy during remission. At the present time, the major use of the nitrosoureas in multiple myeloma appears to be for patients who enter remission with conventional alkylating-agent therapy and later relapse. BCNU and CCNU are occasionally effective when used as single agents or in combination with other alkylating agents for relapsing patients. Results of a pilot study at the University of Arizona with low doses of BCNU and adriamycin for patients relapsing on alkylating-agent therapy have been encouraging, with a 54% (seven of 13 patients) incidence of CRs and PRs. The use of this combination in conjunction with vincristine and prednisone for relapsing patients is under investigation by the Southwest Oncology Group.

[Cancer Treat Rep 60:789–794, 1976]

The use of alkylating-agent therapy for multiple myeloma has resulted in a major improvement in the overall prognosis for patients with this disease. Whereas the median survival in patients who were observed prior to the time of such therapy (and in patients initially refractory to chemotherapy) is reported to be in the range of 8–12 months (1), in most clinical studies with melphalan and prednisone regimens the median survival time is approximately 36 months if a 75% tumor regression is achieved and the overall median survival in myeloma is currently approximately 24 months. Because myeloma has a low-growth fraction (2–4) and is known to be sensitive to more conventional alkylating agents (such as

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[2]Section of Hematology and Oncology, Department of Internal Medicine, University of Arizona College of Medicine, Tucson, and the Southwest Oncology Group (SWOG).

[3]Much of the data reviewed in this paper was abstracted from ongoing clinical trials. I would like to especially acknowledge the following investigators or cooperative group myeloma committee chairmen who assisted in the collation of their data and who permitted it to be reviewed prior to formal publication: *David Alberts* (University of Arizona, Tucson); *Raymond Alexanian* (M.D. Anderson Hospital and Tumor Institute, Houston, Tex, SWOG); *Daniel Bergsagel* (Princess Margaret Hospital, Toronto, Canada, National Cancer Institute of Canada); *John Bonnet* (Scott and White Clinic, Temple, Tex, SWOG); *Harvey Cohen* (Veterans' Administration Hospital, Durham, NC, Southeastern Cancer Study Group [SEG]); *Brian Durie* (University of Arizona, Tucson, SWOG); *Robert Kyle* (Mayo Clinic, Rochester, Minn, Acute Leukemia Group B [ALGB]); *Burton Lee* (Memorial Hospital for Cancer and Allied Diseases, New York, NY); *Robert Mass* (Veterans' Administration Hospital, Portland, Ore, Western Cancer Study Group); *O. Ross McIntyre* (Hitchcock Hospital, Hanover, NH, ALGB); *Albert Schilling* (Boston City Hospital, Boston University Hospital, Mass, Eastern Cooperative Oncology Group); and *Harold Silberman* (Duke University School of Medicine, Durham, NC, SEG).

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melphalan and cyclophosphamide) and to at least one other cycle-nonspecific agent (adriamycin) (5–8), studies of the nitrosoureas in this disseminated neoplasm have appeared rational. Specific protocols for the use of the nitrosoureas have been developed by a number of cooperative groups and individual institutions. Most of the nitrosourea clinical trials have yet to be published. However, investigators have provided summaries of their data and these are presented in this review.

The nitrosoureas have been investigated in two major and quite different settings in myeloma. The first of these is as a component of initial remission-induction therapy, generally in combination with prednisone alone or prednisone and other alkylating agents. The second application in the treatment of refractory myeloma is distinctly different and includes at least two patient categories: (*a*) those who fail to respond to an initial standard induction regimen (usually consisting of melphalan and/or cyclophosphamide plus prednisone), and (*b*) those who have late relapses on such standard regimens after a good initial remission lasting 1–4 years. Many patients in this late relapse subgroup appear to have an accelerated phase of disease that is not too different from the blast crisis of chronic myelogenous leukemia. Studies of treatment for such refractory patients have generally used nitrosoureas in combination with other agents.

REMISSION-INDUCTION THERAPY

Both BCNU and CCNU have been studied alone and in combination with prednisone and alkylating agents for remission induction in previously untreated patients with multiple myeloma. The major dose schedules of the treatment regimens used by various investigators and cooperative groups are summarized in table 1.

Major investigations of nitrosoureas used alone or in combination with prednisone have been carried out by the Acute Leukemia Group B (ALGB) which has studied BCNU given weekly or every 6 weeks and BCNU or CCNU given every 6 weeks alone or in combination with prednisone.[4] In part on the basis of data from these studies, the ALGB has mainly used BCNU at 6-week intervals with prednisone. In these studies, the response rate by ALGB criteria was 40% for BCNU plus prednisone and 44% for a concurrent melphalan-plus-prednisone arm, with an overall median survival of approximately 30 months in both treatment groups for responsive patients. Among "good-risk" patients median survival was approximately 36 months, whereas among "poor-risk" patients median survival was 20 months with both treatment arms.[4] In this experience, more cumulative bone marrow toxicity was observed with the nitrosoureas than with melphalan. Although neither treatment arm is clearly superior, the simplicity, lack of cumulative toxicity, and low cost of oral administration of melphalan plus prednisone make it preferable to BCNU plus prednisone.

Several studies have used combinations of BCNU with other alkylating agents and prednisone for previously untreated myeloma cases. The ongoing joint study by the Southeastern Cancer Study Group (SEG) and the Eastern Cooperative Oncology Group (ECOG) of the combination of BCNU, cyclo-

[4]Leone L. Oral presentation. Myeloma Symposium, Atlanta, Ga, 1974.

TABLE 1.—BCNU-containing combinations used by major single institutions or cooperative groups for remission induction in previously untreated patients with multiple myeloma

Drug (route)	Protocol					
	ALGB Nos. 6701, 6803 (q6wks)	ALGB No. 7261 (q6wks)	Memorial Hospital M–2 (q5wks)	NCI–C (q4wks)	SEG No. 343 and ECOG (q4wks)	SWOG No. 7305* (q4wks)
	Total dose: mg/m²/course					
BCNU (iv)	150	100	18	50	75	30
Melphalan (iv or oral)	—	8†	37	16	—	16
Cyclophosphamide (iv or oral)	—	300†	370	108†	400†	300
Prednisone (oral)	900‡	900‡	550	230	*130*§	240
Vincristine (iv)	—	—	0.6	—	—	—

**See* ref 11.

†Iv administration.

‡Prednisone administered in tapering doses only during initial 10 wks of therapy.

§Prednisone dosage is expressed in mg/m² for comparative purposes using the average normal body surface area (BSA). However, it should be pointed out that the SEG and the ECOG use a constant dose of 75 mg/day × 7 days (total of 525 mg/course) irrespective of weight or BSA.

phosphamide, and prednisone thus far shows no superiority with regard to median survival compared to a simultaneous melphalan plus prednisone control arm (9). In the current Canadian National Cancer Institute (NCI-C) study comparing melphalan plus prednisone (group A) with a combination of melphalan, cyclophosphamide, and BCNU plus prednisone administered alternately (group B) or concurrently (group C), the results thus far have been identical with all three treatment programs with regard to response rates and survival (Bergsagel D, personal communication). In the current ALGB study of melphalan, cyclophosphamide, BCNU, and prednisone versus melphalan and prednisone there may be slightly better survival with the four-drug combination (McIntyre OR, personal communication). However, in the Southwest Oncology Group (SWOG) study, simultaneous administration of melphalan, cyclophosphamide, BCNU, and prednisone has been shown to be inferior with regard to the speed of remission induction in comparison with the concurrent control arms of melphalan, adriamycin, and prednisone and melphalan, cyclophosphamide, and prednisone, as well as a historical melphalan plus prednisone control arm. Only the relatively limited experience of the Memorial Hospital for Cancer and Allied Diseases with the M-2 protocol, which uses the smallest dose of BCNU of any of these studies (as well as the highest doses of melphalan, cyclophosphamide, and prednisone plus vincristine), shows any clear evidence of superiority of the combination over their historical control arm of melphalan plus prednisone (Lee B, personal communication) (10). The survival of patients on the M-2 protocol appears similar to that achieved in the current SWOG study, with a projected median survival > 30 months in responsive patients (10,11). A similar regimen has also been used by Azam and Delamore (12). The ALGB, SWOG, and NCI-C trials also appear to suggest that BCNU used alone or with other alkylating agents is of little value as a crossover treatment for patients who fail to enter remission on other regimens.

Thus, in summary, BCNU appears to add little benefit to other alkylating agents for remission induction for previously untreated patients. It remains to be established whether BCNU in combination with nonalkylating agents such as adriamycin can be better than or equal to other alkylating-agent combinations for initial remission induction.

REMISSION MAINTENANCE

According to a SWOG study chaired by Alexanian (13), remission maintenance with BCNU or melphalan for patients who enter "CR" (> 75% tumor regression) does not add to the overall survival. This result was obtained in a SWOG study that showed remission duration and survival to be statistically identical in patients randomly assigned to melphalan plus prednisone, BCNU plus prednisone, or no further treatment. Furthermore, there was a somewhat higher incidence of infectious complications in the patients receiving continued chemotherapy. I personally do not favor discontinuation of therapy during remission in the following circumstances: (*a*) in patients without a serum M-component (cases of Bence Jones proteinuria only or no M-component); (*b*) in patients with < 75% tumor regression (PR) after induction therapy; (*c*) in patients for whom serum M-component production rate is not precisely followed; and (*d*) in patients with initial high tumor cell mass and residual M-component (although considered to be in remission [> 75% tumor regression]). At any rate, it is clear at least that BCNU offers no superiority over melphalan plus prednisone or no treatment for remission maintenance.

THE RELAPSE PHASE

Patients with myeloma who relapse after initial remission induction with standard alkylating agents represent the population in which nitrosourea combinations may prove most useful. Usually, many such patients have already been shown to be sensitive to cycle-nonspecific agents and introduction of non-cross-resistant agents with similar mechanisms of action appears reasonable. Trials in this category of patients are summarized in table 2 in terms of the best possible results (response in evaluable patients). (Caution is urged in the interpretation of these data since the patient populations are small and heterogeneous and the criteria for response are not uniform.)

Single-agent therapeutic regimens with BCNU, CCNU, or methyl-CCNU have not had activity anywhere near that observed by Young et al (14) for BCNU in patients with stage IV Hodgkin's disease who relapsed after MOPP therapy.[5] Although most specialists in myeloma therapy have seen an occasional dramatic response to single-agent nitrosourea therapy in this setting, the overall PR rate with this approach is no more than 5%–10% (15). In similar circumstances, a pilot study using adriamycin in modest doses induced a 20%–30% PR rate and appeared to be of some clinical value (8).

Recently, at the University of Arizona we have used the two-drug combination of BCNU and adria-

[5]MOPP = mechlorethamine, vincristine, prednisone, and procarbazine.

TABLE 2.—Regimens used in major single-institution or cooperative-group studies for relapsing myeloma patients with nitrosoureas alone or in combination with other drugs

Drug (route)	Protocol				
	ALGB No. 7361 (q6wks)	SEG No. 245 (q6wks)	WCSG* (q6wks)	University of Arizona (q3wks)	SWOG No. 7432 (q3wks)
	Total dose: mg/m²/course				
BCNU (iv)	100	—	—	30	30
CCNU (oral)	—	—	130	—	—
Methyl-CCNU (oral)	—	50 → 200†	—	—	—
Melphalan (iv or oral)	—	—	—	—	—
Cyclophosphamide (iv)	300	—	—	—	—
Prednisone (oral)	730‡	*350*§	—	—	240
Vincristine (iv)	—	—	—	—	0.6
Adriamycin (iv)	—	—	—	30	30
No. of improvements + "PRs"/No. of evaluable patients¶	2/17 (12)	6/33 (18)	0/10 (0)**	7/13†† (54)	8/19 (42)

*Western Cancer Study Group.
†Escalated by 50 mg/course if possible from the starting dose of 50 mg/m² to a maximum of 200 mg/m² by the 4th course.
‡Prednisone administered 1st course only.
§Prednisone dosage is expressed in mg/m² for comparative purposes using the average normal body surface area (BSA). However, it should be pointed out that the SEG and the ECOG use a constant dose of 75 mg/day × 7 days (total of 525 mg/course) irrespective of weight or BSA.
¶Response for each regimen based on criteria established by particular institution or cooperative group.
**See ref 15.
††See ref 16.

mycin in small doses every 3–4 weeks and have observed CRs or PRs in seven of 13 patients (16). Two of the seven had > 75% reductions in tumor cell mass with clinical improvement (CR) and the other five had > 50% reductions in tumor cell mass and clinical improvement (PR). In three of the patients (two with CR and one with PR), remission duration was > 12 months. Several of these patients tolerated this therapy (with blood component support) even when they started treatment in relapse with a white blood cell count of < 1000 cells/mm³ and a platelet count of < 20,000 platelets/mm³.

As a result of these observations with BCNU plus adriamycin the SWOG has undertaken a study of the same BCNU plus adriamycin schedule along with vincristine and prednisone (VBAP) for refractory myeloma. Although the results are still quite preliminary, VBAP also appears useful for the induction of at least PRs in cases of refractory myeloma (table 2). In view of the clear activity of BCNU plus adriamycin in end-stage cases, it would appear that this combination of a DNA "crosslinker" (BCNU) and a DNA binder (adriamycin) may prove to be synergistic or potentiating. Clinical trials of this combination are also warranted in patients who show initial resistance to alkylating agents as well as for primary induction therapy.

COMMENTS

I would like to emphasize that several points should be kept in mind in considering these preliminary results with nitrosoureas in the treatment of multiple myeloma.

First, because normal bone marrow function is commonly impaired in patients with multiple myeloma, the doses of nitrosoureas have been significantly lower than those used for other tumors (eg, brain tumors). Thus, although toxicity probably has been less severe in patients with myeloma, response may also have been limited in view of the rather clear dose-response relationship which occurs with other cycle-nonspecific agents such as melphalan in myeloma (17).

Second, because the various clinical study groups have not yet agreed upon common criteria of response in myeloma, attempts at tabular summaries of results (especially response) have unfortunately necessarily included a number of noncomparable results. Inasmuch as a simple but precise means is now available for quantitation of tumor cell mass and clinical staging of myeloma patients from clinical features of the disease (hemoglobin, serum calcium, and creatinine levels, M-component levels, and bone lesions) (18) and for quantitative followup

of reduction in tumor cell mass from M-component production data (17,19), it is my hope that such quantitative techniques will be applied retrospectively to these trials (20) since they would permit direct comparison of the rate and magnitude of tumor cell-kill results.

In the SWOG experience with patients studied in this manner approximately 45% of the patients have high tumor cell mass (> 1.2×10^{12} myeloma cells; stage III), 29% have intermediate tumor cell mass ($0.6–1.2 \times 10^{12}$ myeloma cells; stage II), and 23% have low tumor cell mass (< 0.6×10^{12} myeloma cells; stage I) at the time of diagnosis.[6] Overall survival is contingent on three major factors: tumor cell mass at the time of diagnosis, renal function, and response to chemotherapy (18). Although advanced stage (III) and impaired renal function both have major effects on survival, complete response to treatment can override these adverse factors (18,20). Because of the extreme importance of accurately staging patients with myeloma, the basic staging system is shown in table 3. Figure 1 shows a simple graphic technique for correcting for M-component metabolism in cases of IgG myeloma. This correction is not required in IgA cases. (I am now preparing a more accurate and simple technique for correcting for metabolism with the use of a programmable pocket calculator.) On the basis of current data the SWOG requirement for CR in patients with Bence Jones proteinuria only is the complete clearing of M-component from the urine on electrophoresis (or at least a reduction to < 200 mg/24 hrs).

Despite these major reservations about less quantitative techniques, I do think that there is sufficient evidence to indicate that BCNU is active in multiple myeloma and that it can be of use in combination therapy for remission induction or in late relapses. It is in the latter application that BCNU would appear to have the greatest utility at the present time.

[6]Minutes of the SWOG Meeting, Houston, Tex, June 1976.

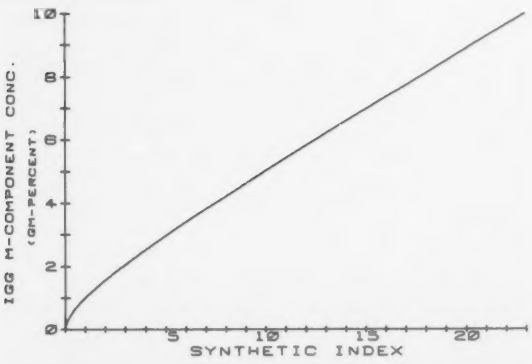

FIGURE 1.—This nomogram is useful for clinical evaluation of tumor cell mass response in IgG myeloma. It is applicable to all IgG subclasses and corrects for concentration-dependent changes in the IgG fractional catabolic rate. Percentage change in tumor cell mass can be calculated directly from percentage changes in the synthetic index shown on the abscissa. A constant plasma volume (3.5 liters) has been assumed. If a patient's plasma volume has been measured serially, appropriate corrections can be made in the synthetic index. For specialized research purposes, synthetic index = total body production rate for IgG (g/24 hrs) divided by 2.0651 (a constant used for normalization of the graph). Total body production rate for IgG = fractional catabolic rate × plasma volume (liters) × IgG concentration (g/liter on electrophoresis) (17,19).

TABLE 3.—Myeloma staging system*

Stage	Criteria	Measured myeloma cell mass (cells × $10^{12}/m^2$)†
I	*All of the following:* 1. Hemoglobin value > 10 g/100 ml 2. Normal serum calcium value (≤ 12 mg/100 ml) 3. Normal bone structure (scale 0) or solitary bone plasmacytoma only on roentgenogram 4. Low M-component production rates a. IgG value < 5 g/100 ml b. IgA value < 3 g/100 ml c. Urine light chain M-component on electrophoresis < 4 g/24 hrs	< 0.6 (low)
II	*Fitting neither stage I nor III*	0.6–1.20 (intermediate)
III	*One or more of the following:* 1. Hemoglobin value < 8.5 g/100 ml 2. Serum calcium value > 12 mg/100 ml 3. Advanced lytic bone lesions (scale 3) 4. High M-component production rates a. IgG value > 7 g/100 ml b. IgA value > 5 g/100 ml c. Urine light chain M-component on electrophoresis > 12 g/24 hrs	> 1.20 (high)

Subclassification: A = relatively normal renal function (serum creatinine value < 2.0 mg/100 ml)‡; B = abnormal renal function (serum creatinine value ≥ 2.0 mg/100 ml)

Examples: Stage IA = low cell mass with normal renal function; Stage IIIB = high cell mass with abnormal renal function

*Adapted from table 7 in ref 18.

†10^{12} cells = approximately 1 kg or 2.2 lbs.

‡If the serum creatinine value is not available, the blood urea nitrogen (BUN) value may be used as an indicator of renal function. (A BUN value of 30 mg/100 ml is roughly equal to a serum creatinine value of 2 mg/100 ml.)

LIST OF COMPOUNDS

Adriamycin: NSC-123127; CAS reg. No. 23214-92-8

BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-

CCNU: NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-

Cyclophosphamide: NSC-26271; CAS reg. No. 6055-19-2; 2*H*-1,3,2-oxazaphosphorine, 2-[bis(2-chloroethyl)amino]tetrahydro-, 2-oxide, monohydrate

Methyl-CCNU: NSC-95441; CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-

Melphalan: NSC-8806; CAS reg. No. 3223-07-2; alanine, 3-[p-[bis(2-chloroethyl)amino]phenyl]-, monohydrochloride, L-

Prednisone: NSC-10023; CAS reg. No. 53-03-2; pregna-1,4-diene-3,11,20-trione, 17,21-dihydroxy-

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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Clinical Studies With Nitrosoureas in Various Solid Tumors

Milan Slavik[1,*]

While the role of the nitrosoureas in malignant gliomas, gastrointestinal cancer, malignant melanoma, bronchogenic carcinoma, malignant lymphomas, and multiple myeloma has been extensively discussed in previous papers in this issue, this presentation will be limited to the clinical studies of the nitrosoureas in adenocarcinoma of the breast, squamous cell carcinoma of the head and neck, sarcomas, and genitourinary tract and gynecologic cancer. The results are from the published and unpublished reports filed with the Investigational Drug Branch, Cancer Therapy Evaluation Program, DCT, NCI, and represent an update of our previous reviews (1–3). The criteria for evaluable patients, and the definitions and calculations of CR and PR rates used in this review have been previously described (1).

In the treatment of breast cancer neither of the three nitrosoureas reviewed have been found significantly useful. Table 1 shows the results of two studies using BCNU as a single agent in patients resistant to previous hormonal therapy and chemotherapy.

The Mayo Clinic study (4) was disappointing because of the lack of responses, and the study conducted by the Southwest Oncology Group (SWOG) (5) has shown only short-term PRs which apparently did not significantly prolong survival. Low PR rates which were generally of short duration have been reported in a number of clinical studies with CCNU (6–12)[2–4] and are summarized in table 1. Methyl-CCNU appears to be inferior to CCNU based on the results of a controlled randomized study of the SWOG (8). The lack of activity of methyl-CCNU in breast cancer is apparent from the results of five studies (8,13,14)[4,5] summarized in table 1. Since there are drugs that are more active in breast cancer, the nitrosoureas are not used in combination chemotherapy for this disease.

In squamous cell carcinoma of the head and neck BCNU has shown activity in a phase I study (15), subsequently confirmed by a Central Oncology Group (COG) study (table 2).[6] CCNU also showed some activity in broad phase II studies conducted by the COG (16) and others (17,18), while four studies (6,19,20)[6] have reported no responses in a small number of patients (table 2). Methyl-CCNU appears to be moderately active based on limited results from reported studies (14,21,22)[5] (table 2). The comparison of the response rates of the three nitrosoureas in squamous cell carcinoma of the head and neck in 159 evaluable patients (table 2) indicates moderate activity for all three drugs, which will be studied further.

In the treatment of sarcomas (table 3), BCNU was the only nitrosourea reported with some responses (15,23),[6] primarily in Ewing's sarcoma. CCNU (6,12,16,18,19)[3,4] and methyl-CCNU (14,21,22,24)[4,5,7] have not revealed any activity in soft tissue sarcomas.

In genitourinary tract malignancies data exist only for renal cell and testicular tumors. Early results (15)[6] with BCNU in renal cell carcinoma unfortunately were not followed with BCNU in subsequent phase II studies since CCNU was studied more closely (table 4). However, the activity originally reported from the Roswell Park Memorial Institute (25) was not confirmed by any of the other studies (16,18,19).[3] Methyl-CCNU did not reveal any activity in renal cell carcinoma in two broad phase II studies (15)[4] (table 4).

In testicular tumors there are not sufficient data for evaluation of BCNU (15),[6] CCNU (16,18),[3] or methyl-CCNU (14)[5] (table 5).

In gynecologic tumors (table 6) there are inadequate data for evaluation of BCNU in both ovarian (26)[6] and cervical (15) cancer, although it appears that CCNU may be active in both tumors. Methyl-CCNU is inactive in ovarian cancer (14,21,22)[5] and suggests moderate activity in cervical cancer (14). However, since most of these results came from

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[2]Krakoff IH. Personal communication, Apr 1976.

[3]Solomon J, and Bateman J. A phase II evaluation of CCNU. Personal communication, 1976.

[4]Martz G, Maurice P, and Holland J. Personal communication, July 1974.

[5]Silberman H, and Huguley CM. Personal communication, Mar 1976.

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[6]COG. Unpublished results.

[7]Hahn G. Personal communication.

TABLE 1.—Nitrosoureas in the treatment of breast cancer

Nitrosourea	Investigator	Ref or footnote No.	No. of patients evaluated	No. of responses	% response	Duration of response (wks)
BCNU	Ahmann	4	16	0	0	
	Bonnet	5	66	19	28.7	12*
Subtotals			82	19	23.1	
CCNU	Hoogstraten	6	36	7	19	14–50+
	Ahmann	7	21	2	10	14†
	Gottlieb	8	29	4	14	12†
	Cunningham	9	27	3	11	24*
	Cruz	10	30	2	7	12.5†
	Krakoff	footnote 2	23	2	9	6–8+
	Solomon	footnote 3	6	0	—	—
	Band	11	18	3	16	—
	Israel	12	4	0	0	—
	Martz	footnote 4	12	0	0	—
Subtotals			206	24	11.6	6–50+
Methyl-CCNU	Ahmann	13	22	1	5	33
	Gottlieb	8	31	1	3	6
	Tranum	14	21	1	5	—
	Silberman	footnote 5	17	0	0	—
	Martz	footnote 4	10	1	10	—
Subtotals			101	4	3.9	6–33
Totals			389	47		

*Mean duration of response.
†Median duration of response.

TABLE 2.—Nitrosoureas in the treatment of squamous cell carcinoma of the head and neck

Nitrosourea	Investigator	Ref or footnote No.	No. of patients evaluated	No. of responses	% response	Duration of response (wks)
BCNU	DeVita	15	13	2 (1 CR)	15.4	10,19
	COG 620	footnote 6	12	2 (1 CR)	16.6	—
Subtotals			25	4 (2 CRs)	16.0	
CCNU	DeConti	19	3	0	0	—
	Broder	20	5	0	0	—
	Hoogstraten	6	5	0	0	—
	Solomon	footnote 3	8	0	0	—
	Cruz	16	28	4	14	5*
	Lindell	17	2	1	50	
	Hoogstraten	18	7	1	14	
Subtotals			58	6	10.3	
Methyl-CCNU	Gailani	21	6	1	17	5
	Firat	22	11	2	18	—
	Silberman	footnote 5	27	2 (1 CR)	7	—
	Tranum	14	29	5	17	—
Subtotals			76	10 (1 CR)	13	
Totals			159	20 (3 CRs)	12	

*Median duration of response.

either broad phase II or disease-oriented studies with patients heavily pre-treated with combination chemotherapy, the activity of the nitrosoureas in cervical cancer can be considered encouraging and should be further explored.

To summarize, the results of clinical studies of three nitrosoureas, BCNU, CCNU, and methyl-CCNU in seven solid tumors were reviewed (table 7).

In adenocarcinoma of the breast none was found useful in combination therapy, although in squamous cell carcinoma of the head and neck and cervix, further studies are needed to confirm their single-agent activity. The nitrosoureas are inactive in soft tissue sarcomas and renal cell carcinoma. There are inadequate data for evaluation for all three in testicular cancer. The paucity of data on BCNU as well as the lack of results from comparative randomized studies reveal the difficulty in adequate testing of analogs sequentially introduced in clinical studies.

TABLE 3.—Nitrosoureas in the treatment of sarcomas

Nitrosourea	Investigator	Ref or footnote No.	No. of patients evaluated	No. of responses	% response	Duration of response (wks)
BCNU	DeVita	15	1	1 (CR)		> 63
	Palma	23	12	5 (1 CR)	42	25
	Cortner	*	6	1	16	—
	Sutow	†	6	1	16	—
	COG 620	footnote 6	16	2	12	—
	WCG	‡	14	3	21	—
Subtotals			55	13 (2 CRs)	23 (4)	
CCNU	Cruz	16	24	2	8	10–26+
	Hoogstraten	6, 18	12	0	0	—
	Israel	12	1	1		6
	DeConti	19	2	0	0	—
	Martz	footnote 4	9	1	11	—
	Solomon	footnote 3	7	0	0	—
Subtotals			55	4	7	
Methyl-CCNU	Young	24	5	0	0	
	Gailani	21	3	1	33	
	Hahn	footnote 7	12	0	0	
	Silberman	footnote 5	15	0	0	
	Martz	footnote 4	6	0	0	
	Firat	22	4	1	25	
	Tranum	14	70	5	7	
Subtotals			115	7	7	
Totals			225	24	9	

*Cortner JA. Unpublished data, 1965.
†Sutow WW. SWOG protocol No. 366. Unpublished data.
‡Protocol No. 107. Unpublished data.

TABLE 4.—Nitrosoureas in the treatment of renal cell carcinoma

Nitrosourea	Investigator	Ref or footnote No.	No. of patients evaluated	No. of responses	% response	Duration of response (wks)
BCNU	DeVita	15	7	2	28	2,4
	COG 620	footnote 6	3	0	0	—
Subtotals			10	2	20	2,4
CCNU	Mittelman	25	20	4	20	12–24
	DeConti	19	1	0	0	—
	Solomon	footnote 3	12	0	0	—
	Hoogstraten	18	9	0	0	—
	Cruz	10,16	18	0	0	—
Subtotals			60	4	6	12–24
Methyl-CCNU	Silberman	footnote 5	29	0	0	
	Tranum	14	16	0	0	
Subtotals			45	0	0	
Totals			115	6	5	2–24

TABLE 5.—Nitrosoureas in the treatment of testicular tumors

Nitrosourea	No. of patients evaluated	No. of responses	% response	Duration of response (wks)
BCNU	8	2	25	3, > 4
CCNU	14	0	0	—
Methyl-CCNU	7	0	0	—
Totals	29	2		

TABLE 6.—Nitrosoureas in the treatment of gynecologic tumors

Nitrosourea	Investigator	Ref or footnote No.	No. of patients evaluated	No. of responses	% response	Duration of response (wks)
		Ovarian cancer				
BCNU	Godfrey	26	11	0	0	
	COG 620	footnote 6	1	1 CR	—	
Subtotals			12	1 CR	8 (CR)	
CCNU	DeConti	19	1	1	—	25
	Hoogstraten	18	10	2	20	—
	Cruz	16	27	7 (2 CRs)	26	10,* 19†
	Omura	‡	11	0	0	—
	Solomon	footnote 3	5	0	0	—
Subtotals			54	10 (2 CRs)	18.5	10–25*
Methyl-CCNU	Gailani	21	2	0	0	
	Silberman	footnote 5	3	0	0	
	Firat	22	4	0	0	
	Omura	‡	10	0	0	
	Tranum	14	22	0	0	
Subtotals			41	0	0	
Totals			107	11		
		Cervical cancer				
BCNU	DeVita	15	1	1 CR	—	> 4
CCNU	DeConti	19	3	1	33	
	Lindell	17	2	0	0	
	Hoogstraten	18	7	2	29	
	Omura	‡	18	3 (1 CR)	16 (6 CRs)	
Subtotals			30	6 (1 CR)	20 (3 CRs)	
Methyl-CCNU	Silberman	footnote 5	2	0	0	
	Firat	22	4	0	0	
	Omura	‡	18	1	5	
	Tranum	14	31	4	13	
Subtotals			55	5	9	
Totals			89	12		

*Median for PR.
†Median for CR.
‡Omura G. Personal communication, 1975.

TABLE 7.—Results of clinical studies of nitrosoureas in solid tumors

	Nitrosourea					
	BCNU		CCNU		Methyl-CCNU	
Tumor	No. of patients evaluated	No. of responses (%)	No. of patients evaluated	No. of responses (%)	No. of patients evaluated	No. of responses (%)
Breast	82	19 (23)	206	24 (11)	110	4 (4)
Head and neck	25	4 (16)	58	6 (10)	76	10 (13)
Sarcomas	55	13 (23)	55	4 (7)	115	7 (7)
Ovary	12	1 (8)	54	10 (18)	41	0
Cervix	1	1	30	6 (20)	55	5 (9)
Kidney	10	2 (20)	60	4 (6)	45	0
Testis	8	2 (25)	15	0	7	0

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Pharmacology of Chlorozotocin (NSC-178248), a New Nitrosourea Antitumor Agent[1]

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SUMMARY

Structure-activity studies of nitrosourea pharmacology have resulted in the synthesis of a new water-soluble agent, chlorozotocin, which has significant antitumor activity against the L1210 leukemia system and produces only a minor degree of inhibition of mouse and human bone marrow DNA synthesis compared to BCNU. It is important to emphasize that the bone marrow sparing feature of chlorozotocin is relative and that if the drug is administered at lethal dose levels in mice, myelosuppression is observed. The potential importance of these studies is the identification of a new and active nitrosourea antitumor agent with modified bone marrow toxicity. If aminoglucose modification of nitrosourea bone marrow toxicity can be confirmed in man without significant loss of antitumor activity, the use of such a compound could facilitate treatment of patients with neoplastic disease who have pre-existing abnormal bone marrow function. It would also allow the more effective use of a nitrosourea agent in combination with anticancer agents possessing more potent myelosuppressive properties.

[Cancer Treat Rep 60:801–805, 1976]

In 1959, 1-methyl-3-nitro-1-nitrosoguanidine (MNNG) was reported to increase the lifespan of mice bearing L1210 leukemia (1). Although MNNG did not have sufficient activity to warrant extensive clinical testing, the demonstration of antitumor effect gave impetus for further evaluation of the *N*-nitroso class of compounds as potential antineoplastic agents. In 1961, 1-methyl-1-nitrosourea (MNU) was found to have antitumor activity against ip implanted L1210 that was superior to that of MNNG (2). In addition, MNU was demonstrated to cross the blood-brain barrier and produce limited but reproducible antitumor activity in mice bearing intracerebrally implanted leukemic cells. Since that time, > 300 alkyl nitrosourea compounds have been screened for antitumor activity, the majority of the synthetic work having been undertaken by Montgomery, Johnston, and co-workers at the Southern Research Institute in Birmingham, Ala. A major emphasis has been directed toward the development of halogenated ethyl derivatives after BCNU was shown to have curative antitumor activity in mice implanted either ip or intracranially with L1210 leukemia. As a group, the chloroethyl nitrosoureas BCNU, CCNU, and methyl-CCNU are now recognized as an important class of clinical antitumor agents with activity having been demonstrated against lymphomas, small cell carcinoma of the lung, malignant melanoma, intracerebral glioma, and gastrointestinal cancer when combined with 5-fluorouracil (3–5). However, these agents can produce profound and delayed bone marrow toxicity. Repeated courses of treatment often result in cumulative injury to the bone marrow and the development of a chronic state of myelosuppression.

Concurrent with the developmental work on chloroethyl derivatives there have been extensive laboratory and clinical investigations of the naturally occurring methyl nitrosourea, streptozotocin, a fer-

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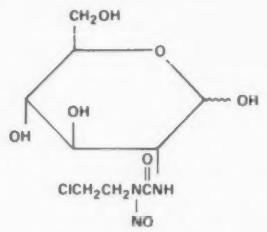
mentation product of *Streptomyces achromogenes*. Although streptozotocin is generally regarded as having less overall clinical usefulness when compared to the chloroethyl compounds, it nevertheless has shown activity against islet cell carcinomas, malignant carcinoid tumors, soft tissue sarcomas, and advanced stages of Hodgkin's disease (6). Of equal interest has been the pharmacologic information derived from the studies of streptozotocin, which may ultimately make a significant impact upon future developments for the entire class of alkyl nitrosourea compounds.

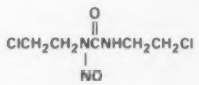
Streptozotocin is composed of MNU attached to an aminoglucose carrier (7). The compound has produced two unique pharmacologic actions in experimental systems. The first is the ability to selectively destroy the pancreatic β cell of the islet of Langerhans in animals which results in a permanent diabetic state. Studies of the biochemical basis of this diabetogenic activity have shown it to be mediated through a rapid reduction in pyridine nucleotide concentrations, an observation initially demonstrated in the liver and subsequently confirmed in isolated pancreatic islets (8–11). The second unique feature of streptozotocin's pharmacology is its relative lack of myelosuppressive activity while retaining antileukemic activity in the L1210 and P388 tumor systems (8). This ability to spare the normal bone marrow has been confirmed in clinical trials and streptozotocin can now be safely used in patients with depressed bone marrow function (6). In contrast, the MNU cytotoxic group of the streptozotocin molecule is a significant bone marrow toxin in animals (12). This raised the question of whether the aminoglucose carrier was in some way responsible for the reduced myelosuppressive activity of streptozotocin.

To further evaluate the potential importance of the aminoglucose carrier, two new compounds, chlorozotocin and the tetra-acetate form of the same agent, were synthesized for our studies by Johnston et al (13). This has allowed a structure-activity comparison of the relative biologic and biochemical properties of this aminoglucose chloroethyl compound with those of the existing myelosuppressive analogs BCNU (fig 1) and CCNU (14,15). The chemical structure of chlorozotocin is compared to BCNU in figure 1.



2-[3-(2-chloroethyl)-3-nitrosoureido]-2-deoxy-D-glucopyranose

NSC-178248



1,3-bis(2-chloroethyl)-1-nitrosourea

NSC-409962

FIGURE 1.—Chlorozotocin and BCNU.

TABLE 1.—Differential effect of chlorozotocin and BCNU on peripheral wbc counts at nadir (Day 3)

Drug	Ip dose (mg/kg)	Wbc: % of control	Wbc differential (%)			
			Neutrophils	Lymphocytes	Monocytes	Eosinophils
Chlorozotocin	20 (LD10)	72	43	52	5	0
	50 (LD100)	24	72	20	8	0
BCNU	20 (LD0)	60	12	78	8	2
	30 (LD20)	28	19	73	9	0
Control	—	100	44	45	10	1

To evaluate the relative bone marrow toxicity, groups of ten normal BDF_1 mice received single ip doses of chlorozotocin ranging from 10 to 50 mg/kg. Serial peripheral white blood cell (wbc) counts were performed over a 30-day period of observation. The results were compared with wbc counts from mice that had received single ip doses of BCNU (20 or 30 mg/kg) (15) (table 1). The nadir of wbc depression occurred 3 days after administration for both drugs. Chlorozotocin, administered at a maximum nonlethal dose (15 mg/kg), produced an 18% reduction in the mean wbc count which was not significantly different from that of the control. A 10% lethal dose (20 mg/kg) produced a 28% decrease in the wbc count with no specific alteration in the neutrophil/lymphocyte ratio. Administration of a 100% lethal dose (50 mg/kg) decreased the wbc count by 76%, with a preferential reduction in circulating lymphocytes. Histologic examination of the bone marrow of these animals showed only a moderate reduction in the granulocytic series, whereas there was a major decrease in the size of the splenic lymphoid follicles. With the 50-mg/kg dose, all animals died between the fourth and sixth day after injection.

BCNU, administered at a maximum nonlethal dose of 20 mg/kg, produced a 40% reduction in wbc count which was significantly different from that of the control ($P < 0.05$). In contrast to chlorozotocin, wbc differential counts showed a preferential reduction in circulating neutrophils. A 20% lethal dose of BCNU (30 mg/kg) caused a reduction in the wbc count comparable to a 100% lethal dose of chlorozotocin. The bone marrows of mice killed on Day 3 after injection were in a generalized hypoplastic state, whereas the splenic lymph follicles were only moderately reduced in size compared to controls. Animals died between 21 and 30 days after treatment at this dose of BCNU. This is in contrast to the acute mortality observed with chlorozotocin at lethal dose levels. This relative bone marrow sparing property of chlorozotocin has been confirmed by Dr. Leon Schmidt et al at the Southern Research Institute.

Table 2 summarizes the results of studies comparing the relative antitumor activity of chlorozotocin and BCNU in mice implanted ip with 10^5 L1210 leukemia cells. The animals received graded single ip doses of chlorozotocin or BCNU after 2 or 6 days

TABLE 2.—Survival of BDF_1 mice treated on Day 2 and Day 6 of L1210 tumor growth*

Drug	Dose: Mg/kg	Dose: Lethality in normal mice	Average lifespan (days)	%ILS	No. of 90-day survivors/No. of mice treated
		Day 2			
Chlorozotocin	5	—	19.1	140	1/10
	10	—	37.8	355	3/10
	15	—	66.5	701	6/10
	20	LD10	48.7	487	4/10
	30	LD90	38.7	366	3/10
	50	LD100	7.3	—	0/10
Control		—	8.3	—	0/10
BCNU	10	—	23.0	125	1/9
	20	—	55.5	444	5/10
	30	LD20	74.7	632	7/9
	40	LD20	74.7	632	8/10
	50	LD100	49.4	384	4/10
Control		—	10.2	—	0/10
		Day 6†			
Chlorozotocin	10	—	19.2	137	1/10
	15	—	13.4	65	0/10
	20	LD10	40.6	401	3/10
	30	LD90	37.1	358	3/10
Control		—	8.1	—	0/10
BCNU	10	—	17.3	97	0/10
	20	—	25.0	184	1/10
	30	LD20	52.5	493	5/10
	40	LD20	14.9	69	0/10
	50	LD100	23.7	169	1/10
Control		—	8.8	—	0/10

*10^5 L1210 cells ip on Day 1.
†*From* ref 15.

of tumor growth. The maximally effective dose of chlorozotocin against the Day-2 tumor was 15 mg/kg ip, which produced a 701% increase in lifespan (ILS) compared to untreated controls; 60% of the mice survived for 90 days. This dose level was not myelosuppressive in normal mice. The maximally effective dose for BCNU against the Day-2 tumor was 30 mg/kg. This dose resulted in a 632% ILS compared to untreated controls with seven of nine animals alive after 90 days of observation. This dose of BCNU in normal mice had produced a 72% reduction in normal circulating wbcs.

Chlorozotocin at a dose of 20 mg/kg resulted in a 410% ILS in animals treated on Day 6 of tumor growth, with 30% of the animals surviving for 90 days. With BCNU, mice had a 493% maximum ILS, with 50% of the animals surviving for 90 days. Thus, the antitumor activities of chlorozotocin and BCNU in the L1210 leukemia system are similar; both agents demonstrate curative activity in both the early and late stages of L1210 tumor growth.

To correlate the relative bone marrow toxicity and antitumor activity of chlorozotocin and BCNU, measurements of DNA synthesis were performed using mice with 4 days of L1210 tumor growth (15). Chlorozotocin (15 mg/kg) or BCNU (30 mg/kg) was administered ip and 1 hour before being killed mice were given 100 microcuries of ^{3}H-thymidine ip. The ascites tumor cells were aspirated from the peritoneal cavity and the normal bone marrow was expressed with phosphate-buffered saline. DNA was extracted and counted for radioactivity, results being expressed as dpm/μg of DNA. Chlorozotocin (15 mg/kg) produced a 96% inhibition of L1210 DNA synthesis within 24 hours of administration, as measured by ^{3}H-thymidine incorporation into DNA. BCNU (30 mg/kg) resulted in an 85% inhibition at the same time period. Both drugs produced a 94% inhibition in DNA synthesis by 48 hours after treatment. Chlorozotocin did not significantly reduce DNA synthesis of bone marrow. BCNU, however, did reduce the incorporation of thymidine into bone marrow DNA to 37% of the control by 24 hours after treatment. This degree of inhibition was significant ($P < 0.01$). With both drugs, a rebound in DNA synthesis in bone marrow above the control level was observed by 48 hours after administration.

These observations have now been extended to normal human bone marrow. A comparison of the in vitro activity of chlorozotocin and BCNU on bone marrow DNA synthesis was performed to determine whether the differential effect noted in animals could be reproduced in a human system (16). Four milliliters of bone marrow, obtained from iliac crest aspirates of normal volunteers, was incubated for 1 hour with graded concentrations of the two drugs and resuspended in culture media; the cells were pulsed with ^{3}H-thymidine 1 hour before the study. Twenty hours after exposure to BCNU (1×10^{-4} M), bone marrow DNA synthesis was reduced to 58% of control values. BCNU (5×10^{-4} M) reduced the incorporation of ^{3}H-thymidine into DNA to 12% of control values. In contrast, similar concentrations of chlorozotocin failed to inhibit bone marrow DNA synthesis by the 24-hour time period. Chlorozotocin (1×10^{-3} M) produced a 24% reduction in the incorporation of ^{3}H-thymidine. The results of these in vitro studies using human cells closely parallel those of in vivo investigations in mice.

Attempts are now being made to better understand the biochemical mechanisms which produce differences between chlorozotocin and the remaining chloroethyl nitrosoureas. The aqueous decomposition of BCNU and CCNU has been studied. In addition to the formation of an isocyanate, there is also decomposition to alkylating chloroethyl carbonium intermediates (17,18). Wheeler et al (19) have subjected 17 haloethyl nitrosoureas to computer analysis to estimate the relative role of alkylating and carbamoylating activities by the isocyanate group, in determining the toxicity and the antitumor activity against L1210 leukemia in mice. Alkylating activity was found to correlate closely with the relative antitumor activity, whereas carbamoylating activity was suggested as an important contributing mechanism for lethal toxicity (19).

MNU and BCNU have significant carbamoylating activity when measured by in vitro methods involving incubation with ^{14}C-lysine and separation of products with paper electrophoresis. In contrast, streptozotocin and chlorozotocin have carbamoylating activity that is approximately 1% of that of the corresponding methyl- or chloroethyl-nitrosourea analog. In regard to in vitro alkylating activity chlorozotocin is superior to BCNU when the 4-(para-nitrobenzyl)pyridine assay is performed under conditions of 37°C and pH 6.0.

It thus appeared that the reduced myelosuppressive activity of chlorozotocin might relate to the relative difference in carbamoylating activity.

There are current studies on the role of carbamoylating activity for the toxicity of nitrosourea antitumor agents. MNU has been compared to compounds in which the MNU moiety was substituted onto the carbon-1 or carbon-2 position of glucose or galactose. All four amino-sugar analogs demonstrated reduced bone marrow toxicity when compared to MNU at comparable LD10 doses. This could not be correlated with relative carbamoylating activity since the carbon-1-substituted analogs produced in vitro carbamoylation equivalent to that of MNU (20). These results suggest that carbamoylating ac-

tivity per se is not a reliable indicator of myelosuppressive activity.

LIST OF COMPOUNDS

BCNU: NSC-409962; CAS reg. No. 154–93–8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-

CCNU: NSC-79037; CAS reg. No. 13010–47–4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-

Chlorozotocin: NSC-178248; D-glucopyranose, 2-[[[(2-chloroethyl)nitrosoamino]carbonyl]amino]-2-deoxy-

Methyl-CCNU: NSC-95441; CAS reg. No. 33073–59–5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-

MNNG: NSC-9369; CAS reg. No. 70–25–7; guanidine, 1-methyl-3-nitro-1-nitroso-

MNU: NSC-23909; CAS reg. No. 684–93–5; urea, 1-methyl-1-nitroso-

Streptozotocin: NSC-85998

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The Nitrosoureas—Thoughts for the Future

Stephen K. Carter* and Todd H. Wasserman[1]

The nitrosoureas have a long clinical history. In 1959, 1-methyl-3-nitro-1-nitrosoguanidine (MNNG) had a brief but unproductive clinical trial in man after its weak activity against leukemia L1210 was demonstrated in Southern Research Institute studies (Birmingham, Ala) (1). Further analog development yielded 1-methyl-1-nitrosourea (MNU), which has undergone extensive clinical evaluation in the USSR. During synthesis of additional compounds, the 23rd analog of MNU was BCNU which entered clinical trial in the early 1960s. After BCNU, in rapid succession, CCNU entered clinical study in 1969 followed by methyl-CCNU in 1971.

The Cancer Therapy Evaluation Program of the DCT, NCI, has accumulated data on > 3000 patients treated with BCNU, CCNU, and methyl-CCNU as single agents, and a definite spectrum of activity has been established (table 1).

As with any anticancer compound, the nitrosoureas have moved from single-agent use into combination chemotherapy and combined modality approaches developed along disease-oriented lines. At the same time, analog development has continued in an effort to obtain compounds with either superior therapeutic indexes in responsive tumors or activity against previously unresponsive malignancies.

A major problem which faces the clinician who attempts to move the nitrosoureas into combined modality approaches is the lack of specific data identifying one of the compounds as clearly superior to the other compounds for a given tumor type. In a review by Wasserman et al (2) it was shown that there have been only seven direct comparative studies. Only one of these studies compared BCNU and CCNU while six were comparisons of CCNU and methyl-CCNU. A comparative trial of BCNU and methyl-CCNU has not been conducted except for a BCNU plus radiotherapy versus methyl-CCNU plus radiotherapy trial in gliomas. Only two studies have revealed any differences. Both of these were Hodgkin's disease protocols of the Acute Leukemia Group B, which revealed superiority for CCNU over BCNU and for CCNU over methyl-CCNU (2,3).

Further analysis of the clinical data shows that 16 sequential trials have been performed (2). Of these, three have shown potentially meaningful differences. In studies of Hodgkin's disease by the Southeast Oncology Group, an early trial (4) of BCNU yielded a response rate of 40% (25 of 63 patients) while a much later trial of methyl-CCNU gave a disappointing response rate of 6% (one of 17 patients) (2). This can be explained to some extent by the fact that prior treatment was probably much more extensive in the later trial than in the earlier one which pre-dated the use of MOPP therapy (mechlorethamine, vincristine [Oncovin], prednisone, and procarbazine) by that particular group of investigators.

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TABLE 1.—Summary of activity of single nitrosoureas in various tumors*

Tumor type	No. of responses/evaluable patients (%)		
	BCNU	CCNU	Methyl-CCNU
Brain†	67/142 (47)	47/115 (41)	12/44 (27)
Hodgkin's disease	94/213 (44)	40/84 (48)	19/74 (26)
Non-Hodgkin's lymphoma	30/107 (28)	13/52 (25)	10/57 (18)
Melanoma	22/123 (18)	17/136 (13)	15/124 (12)
Lung	9/83 (11)	46/279 (16)	33/341 (10)
Breast	16/76 (21)	20/170 (12)	4/100 (4)
Colorectal	17/128 (13)	20/222 (9)	18/168 (11)
Head and neck	4/25 (16)	4/50 (8)	6/53 (11)
Renal cell	—	4/55 (7)	0/26 (0)
Cervix	—	—	4/32 (13)
Totals	259/897 (29)	211/1163 (18)	121/1019 (12)

**From* ref 2.

†Gliomas only: BCNU, 38/85 (45%); CCNU, 37/73 (37%); methyl-CCNU, 3/13 (23%).

In the Southwest Oncology Group all of the nitrosoureas have been studied sequentially in breast cancer. An early study reported a 36% response rate (13 of 36 patients) for BCNU (5). This study was complicated because hormones were also given to some patients and the extent of prior treatment was significantly less than in later studies; this is a recurrent theme in most sequential study analyses. In a later study (6), CCNU and methyl-CCNU were compared to adriamycin in a randomized trial. CCNU gave a 14% response rate (four of 29 patients) and methyl-CCNU a 3% response rate (one of 31 patients); both agents were clearly inferior to adriamycin. However, the authors did point out that the hematologic toxicity of methyl-CCNU was less than that of the other two drugs, indicating that this nitrosourea may not have been used as aggressively as the others.

At the Mayo Clinic (Rochester, Minn), investigators have extensively studied the nitrosoureas in large bowel cancer. They consider methyl-CCNU to be the best nitrosourea based not only on its single-agent activity but also on its activity in a series of direct comparisons with 5-fluorouracil (5-FU) (7).

When all of the available data are summarized and compared, significant numerical differences between the nitrosoureas are found only in breast cancer and Hodgkin's disease (2). Nevertheless, a kind of clinical consensus has developed which cannot be supported when the great heterogeneity of the data is examined. This consensus states that BCNU is the nitrosourea of choice for brain tumors, CCNU for Hodgkin's disease, and methyl-CCNU for gastrointestinal cancer and malignant melanoma. It is unfortunate, however, that despite the extensive clinical investigation over so many years, data do not exist which can confirm or deny these conclusions. The principal weakness of the clinical data is the lack of a sufficient number of comparative trials in tumors where nitrosoureas have a therapeutic role. These trials are not likely to be performed in the future and probably would not be worthwhile at this point since it is clear that dramatic differences probably do not exist between the three drugs.

One factor that may account for the scarcity of comparative trials has been the successive emergence of "newer" nitrosoureas into clinical trial before the chemotherapeutic role of the older nitrosourea has been fully established. Much of the phase II data were still being established for BCNU when CCNU was introduced, and the same is true for CCNU and methyl-CCNU. There has been a tendency for investigators to move rapidly to study the "newer" nitrosoureas because of the anticipated improved clinical activity predicted by the preclinical data.

A major weakness in using sequential trials and/or summary data for comparison is the lack of assured patient comparability due to the lack of randomization. Some aspects of patient comparability can be met by historical review, but, once again, the chronology of the successive use of nitrosoureas in clinical trials is an important one. The trial of each new nitrosourea in phase II studies involved patients who had had more prior chemotherapy. Because the state-of-the-art in chemotherapy has grown with time and more aggressive (toxic) chemotherapy and more drug combinations have been used, patients have had more advanced disease which was potentially more resistant to chemotherapy as each nitrosourea was introduced.

Despite the weakness of the clinical data, the failure of methyl-CCNU to exceed or even equal the clinical efficacy of BCNU and CCNU raises questions concerning the preclinical results. The prediction of greater activity for methyl-CCNU came from the Lewis lung carcinoma system, a tumor that has been assigned new predictive priorities in the NCI drug screening program (8). Did the experimental tumor system falsely predict for methyl-CCNU activity? A simple "yes" or "no" answer is impossible. The chronology of nitrosourea trials, as discussed above, may be a factor. Also, a parenteral formulation of methyl-CCNU was tested in animal tumors while oral methyl-CCNU was used in clinical studies. On the other hand, oral CCNU in clinical use was not the same as the parenteral form tested in animal systems. Will the newly introduced parenteral methyl-CCNU possess the clinical activity predicted in animals? Many future clinical trials will undoubtedly be needed to answer these questions.

In some respects the preclinical development (analog synthesis and animal tumor testing) and clinical trials of the nitrosoureas have been failures. The goal for a better nitrosourea promised in the prediction of improved activity for methyl-CCNU compared to BCNU and CCNU has not been totally fulfilled. In fact, there are no good data to support the fact that BCNU is better than the original nitrosoureas MNNG and MNU.

MNNG had only a brief clinical trial that was probably inadequate, but investigators in the USSR have studied MNU in some detail (9). They have reported good clinical activity in lung cancer (eight of 48 patients or a 17% response rate for $\geq$ 50% responses), all of the responses being in the undifferentiated or small cell type (eight of 21 patients [38%]), and in Hodgkin's disease (12 of 18 patients or 67% for $\geq$ 50% responses). Thus, there are some data showing that the clinical activity of MNU may be comparable to BCNU, CCNU, and methyl-CCNU.

All of this has great impact upon the future of

conclusive efforts in drug development. Future analogs for clinical use will have to be chosen by criteria other than their activity in systems such as L1210 and Lewis lung. Schein et al (10), in another paper in this issue, have evaluated the selection of chlorozotocin as the next nitrosourea entering clinical study under NCI sponsorship.

To the extent that the nitrosoureas are active in given tumor types, they will find their ultimate use in combined modality studies that are currently being conducted in a variety of tumor types. Presently the most vigorous pursuit of this aim is in gastrointestinal cancer, particularly in large bowel cancer, where methyl-CCNU plus 5-FU is being evaluated in a variety of studies. This work is based on the recently completed study of the Mayo Clinic which indicates that the combination of 5-FU, methyl-CCNU, and vincristine may produce objective responses in advanced colorectal cancer significantly superior to those obtained with 5-FU alone (11). In a controlled study a 43.5% objective response rate was obtained with this combination compared to only 19% with 5-FU alone ($P < 0.05$).

The superiority of the combination of methyl-CCNU plus 5-FU over 5-FU alone has been shown by the Southwest Oncology Group in a randomized study of 271 patients (12). The 5-FU (400 mg/m²/wk iv) was either given alone or combined with methyl-CCNU (175 mg/m² orally q6wks). The response rate to 5-FU alone in large bowel cancer was 14% (five of 36 patients), while 30% (38 of 128 patients) responded to the combination regimen. The combination was more toxic to the bone marrow with a median white blood cell nadir of 3.3×10^3 cells and a platelet nadir of 75×10^3 cells compared to 5.1×10^3 and 155×10^3 cells respectively for 5-FU alone. Einhorn et al (13) have treated 24 large bowel cancer patients with the combination of 5-FU and methyl-CCNU using the fluorinated pyrimidine on a schedule of 800 mg/m² as a continuous 24-hour iv infusion in 1000 ml D_5W for 5 consecutive days. Methyl-CCNU was given orally at a dose of 150 mg/m² on the first day of the 5-FU infusion. Three courses were given at 4-week intervals followed by maintenance with oral weekly 5-FU (500–1000 mg) and methyl-CCNU (125 mg/m² orally) every 4 weeks. Partial remissions occurred in seven of 24 patients (29%), with a median duration of 6 months (range, 3–9+ months). There was minimal toxicity consisting of mild nausea and vomiting and myelosuppression.

The rationale for using chemotherapy as a surgical adjuvant has evolved significantly during the past 15 years. Initially, the use of chemotherapeutic agents was limited to an interoperative or immediately postoperative course in an attempt to sterilize the clonogenic cells liberated by surgical trauma. This empirical approach was based on early observations of circulating tumor cells and on experimental work in animal model systems showing the beneficial antitumor activity of chemotherapy against these cells. However, little consideration was given to the growth kinetics of bowel tumor primaries or metastases, and the drugs used had only a relatively low order of activity in advanced bowel cancer.

Current studies employ chemotherapy for a longer period of time in the hope of eradicating the microscopic foci of metastatic disease remaining after surgery.

The Gastrointestinal Tumor Study Group of the DCT, a contract-supported group of major cancer centers performing combined modality studies in the major gastrointestinal adenocarcinomas, has recently initiated a series of studies in large bowel cancer. In rectal carcinoma (ie, the distal edge of the primary ≤ 12 cm from the anal verge), patients who have undergone a curative resection of Dukes B_2 (extension to perirectal fat) and C carcinoma with no gross tumor remaining will be entered into a study comparing: (*a*) postoperative radiation, (*b*) postoperative chemotherapy involving methyl-CCNU plus 5-FU, (*c*) postoperative radiotherapy and chemotherapy, and (*d*) no additional treatment (fig 1). In patients with colon cancer (> 12 cm from the anal verge), immunotherapy will be substituted for the radiotherapy in an otherwise similar protocol design. The immunotherapy will be the methanol-extracted residue of bacillus Calmette-Guérin.

The Veterans' Administration Surgical Adjuvant Study Group has recently initiated a similar study (protocol 27) of methyl-CCNU and 5-FU, but using only two treatment arms (surgery versus surgery and chemotherapy). The drugs are administered in 5-day courses beginning as soon after surgery as permissible by the patient's condition (about 10–14 days after operation) and repeated at 7-week intervals for 1 year. On the first day, oral methyl-CCNU is given at a dose of 120 mg/m², and on the same day and each of the next 4 days 5-FU is given at 9 mg/kg iv.

The nitrosourea plus 5-FU combination seems even more active in gastric cancer. In a study at the Mayo Clinic, BCNU plus 5-FU gave a 41% response rate with a median duration of response of 7 months. When compared with 5-FU alone, which was another randomized arm in the study, the combination elicited a 27% survival at 18 months compared to only 7% for 5-FU (14).

More recently, the value of the 5-FU-nitrosourea combination in gastric cancer has been confirmed by the Eastern Cooperative Oncology Group (15)

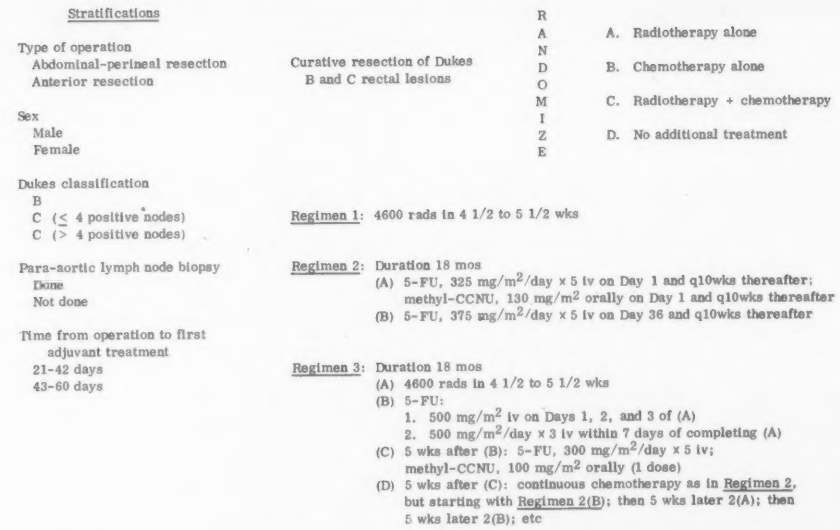
Stratifications
Type of operation
Abdominal-perineal resection
Anterior resection
Sex
Male
Female
Dukes classification
B
C (≤ 4 positive nodes)
C (> 4 positive nodes)
Para-aortic lymph node biopsy
Done
Not done
Time from operation to first adjuvant treatment
21-42 days
43-60 days
Curative resection of Dukes B and C rectal lesions
RANDOMIZE
A. Radiotherapy alone
B. Chemotherapy alone
C. Radiotherapy + chemotherapy
D. No additional treatment
Regimen 1: 4600 rads in 4 1/2 to 5 1/2 wks
Regimen 2: Duration 18 mos
(A) 5-FU, 325 mg/m2/day x 5 iv on Day 1 and q10wks thereafter; methyl-CCNU, 130 mg/m2 orally on Day 1 and q10wks thereafter
(B) 5-FU, 375 mg/m2/day x 5 iv on Day 36 and q10wks thereafter
Regimen 3: Duration 18 mos
(A) 4600 rads in 4 1/2 to 5 1/2 wks
(B) 5-FU:
1. 500 mg/m2 iv on Days 1, 2, and 3 of (A)
2. 500 mg/m2/day x 3 iv within 7 days of completing (A)
(C) 5 wks after (B): 5-FU, 300 mg/m2/day x 5 iv; methyl-CCNU, 100 mg/m2 orally (1 dose)
(D) 5 wks after (C): continuous chemotherapy as in Regimen 2, but starting with Regimen 2(B); then 5 wks later 2(A); then 5 wks later 2(B); etc

FIGURE 1.—Gastrointestinal Tumor Study Group protocol for rectal carcinoma.

BCNU (USA)

CCNU (USA)

Methyl-CCNU (USA)

Streptozotocin (USA)

Chlorozotocin (USA)

MNU (USSR)

ACNU (Japan)

Sugar-modified analogs (France)

PCNU (USA)

FIGURE 2.—Nitrosoureas under clinical evaluation throughout the world.

where a 52% response rate was seen in 23 patients. Currently, methyl-CCNU plus 5-FU is being utilized as an adjuvant to chemotherapy after curative resection in studies of the Gastrointestinal Tumor Study Group and the Veterans' Administration Surgical Adjuvant Study Group.

In bronchogenic carcinoma no nitrosourea has been found to be clearly superior to any other, and all of them as single agents have had little, if any, impact on survival. CCNU has been the most extensively studied of the nitrosoureas and is the one used in combined modality approaches of chemotherapy after curative resection. The Lung Cancer Working Party of the DCT has a protocol for resectable epidermoid disease, as well as resectable large cell carcinoma and adenocarcinoma, in which surgery alone is compared to surgery plus CCNU. The Veterans' Administration Surgical Adjuvant Group has a similar study comparing CCNU plus hydroxyurea after surgery versus surgery alone.

At present, developmental work on the nitrosoureas is continuing throughout the world (fig 2). The NCI is ready to initiate clinical trials of a fifth nitrosourea (chlorozotocin) and recently began development of PCNU which will ultimately be the sixth drug of this class sponsored for trial. Clinical work has just begun in Japan on a new analog, ACNU. As mentioned earlier, MNU has been evaluated in the USSR, and in France a series of sugar-modified analogs are being studied.

It can be hoped that these newer analogs will provide compounds with improved therapeutic indexes. Clearly, the nitrosoureas will be prominent in clinical studies for years to come.

LIST OF COMPOUNDS

ACNU: NSC-245382; CAS reg. No. 55661-38-6; urea,-*N'*-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-*N*-(2-chloroethyl)-*N*-nitroso-, monohydrochloride

Adriamycin: NSC-123127; CAS reg. No. 23214-92-8

BCNU: NSC-409962; CAS reg. No. 154-93-8; urea, 1,3-bis(2-chloroethyl)-1-nitroso-

CCNU: NSC-79037; CAS reg. No. 13010-47-4; urea, 1-(2-chloroethyl)-3-cyclohexyl-1-nitroso-

Chlorozotocin: NSC-178248: D-glucopyranose, 2-[[[(2-chloroethyl)nitrosoamino[carbonyl]amino]-2-deoxy-

5-Fluorouracil: NSC-19893; CAS reg. No. 51-21-8

Hydroxyurea: NSC-32065; CAS reg. No. 127-07-1

Methyl-CCNU: NSC-95441; CAS reg. No. 33073-59-5; urea, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitroso-

MNNG: NSC-9369; CAS reg. No. 70-25-7; guanidine, 1-methyl-3-nitro-1-nitroso-

MNU: NSC-23909; CAS reg. No. 684-93-5; urea, 1-methyl-1-nitroso-

PCNU: NSC-95466; CAS reg. No. 13909-02-9; urea, 1-(2-chloroethyl)-3-(2,6-dioxo-3-piperidyl)-1-nitroso-

Streptozotocin: NSC-85998

Vincristine: NSC-67574; CAS reg. No. 2068-78-2; leurocristine, sulfate (1:1)

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